

Bulletin No. 46 from the Department of Surgery,

Lund University, S-221 85 LUND

1984

HUMAN VASCULAR α -ADRENOCEPTORS

A study of peripheral arteries and veins

in vitro and in vivo



Stig Steen



HUMAN VASCULAR α -ADRENOCEPTORS

A study of peripheral arteries and veins in vitro and in vivo

Stig Steen

leg läkare

Lund

Akademisk avhandling

som för avläggande av medicinsk doktorsexamen
vid Medicinska Fakulteten vid Universitetet i Lund
kommer att offentligens försvaras å Föreläsningssal 4
Centralblocket, Lasarettet i Lund,
lördagen den 16 juni 1984, kl 09.15

Organization LUND UNIVERSITY Department of Surgery Lund University S-221 85 LUND Sweden		Document name DOCTORAL DISSERTATION	
		Date of issue 1984-06-16	
		CODEN: LUMEDW (MESL 1027) /1-182/1984	
Author(s) Stig Steen		Sponsoring organization	
Title and subtitle HUMAN VASCULAR α -ADRENOCEPTORS. A study of peripheral arteries and veins in vitro and in vivo.			
Abstract By means of a sensitive myograph and α-receptor subtype selective agonists and antagonists the postjunctional α-receptors were characterized in small omental and groin arteries and veins and in long saphenous veins obtained from 52 "healthy" adults during surgery. These vessels were found to have a mixed population of contraction mediating postjunctional α-receptors with a predominance of α_1-receptors in the arteries and a predominance of α_2-receptors in the veins, although regional variations occurred. Noradrenaline was 5-10 times more potent in the veins than in the arteries. Changes in diameter of the long saphenous vein <u>in vivo</u> were measured in 6 healthy subjects by means of infrared light and a phototransistor; the α_1-receptor-antagonist prazosin did not significantly antagonize the noradrenaline induced contraction of the saphenous vein in vitro, but was a potent antagonist in vivo whereas the α_2-receptor antagonist yohimbine was potent both in vitro and in vivo. Vessels obtained from the legs of 8 patients with severe ischemia due to atherosclerosis were studied <u>in vitro</u>. The contractile capacity of the arteries was generally poor whereas the veins behaved as vessels from healthy individuals. Eight patients with similar vascular disease were studied <u>in vivo</u>. Noradrenaline caused a more than 70% reduction of femoral artery blood flow in all patients without systemic effects. Yohimbine increased femoral blood flow in five patients, and decreased it in three, whereas prazosin increased it in all patients (by up to 150%). Papaverine increased the femoral blood flow by more than 350%. Prazosin, yohimbine and papaverine had all similar effects on the skin blood flow (measured by laser doppler technique), decreasing it in 6 patients and leaving it unchanged in 2 patients. Epidural anesthesia did not abolish the effects of noradrenaline. In spite of epidural anesthesia prazosin and papaverine increased femoral blood flow by up to 100% and 200% respectively.			
Key words α -Adrenoceptor subtypes, α_1 -adrenoceptors, α_2 -adrenoceptors, human peripheral arteries and veins, atherosclerotic crural arteries, femoral artery blood flow, crural skin blood flow.			
Classification system and/or index terms (if any)			
Supplementary bibliographical information		Language English	
ISSN and key title		ISBN	
Recipient's notes		Number of pages 182	Price
		Security classification	

Distribution by (name and address)

Stig Steen, M.D., Department of Surgery, Lund University, S-221 85 LUND, Sweden

I, the undersigned, being the copyright owner of the abstract of the above-mentioned dissertation, hereby grant to all reference sources permission to publish and disseminate the abstract of the above-mentioned dissertation.

Signature

Stig Steen

Date May 17th, 1984.

From Department of Surgery and
Department of Clinical Pharmacology

Lund University

Sweden

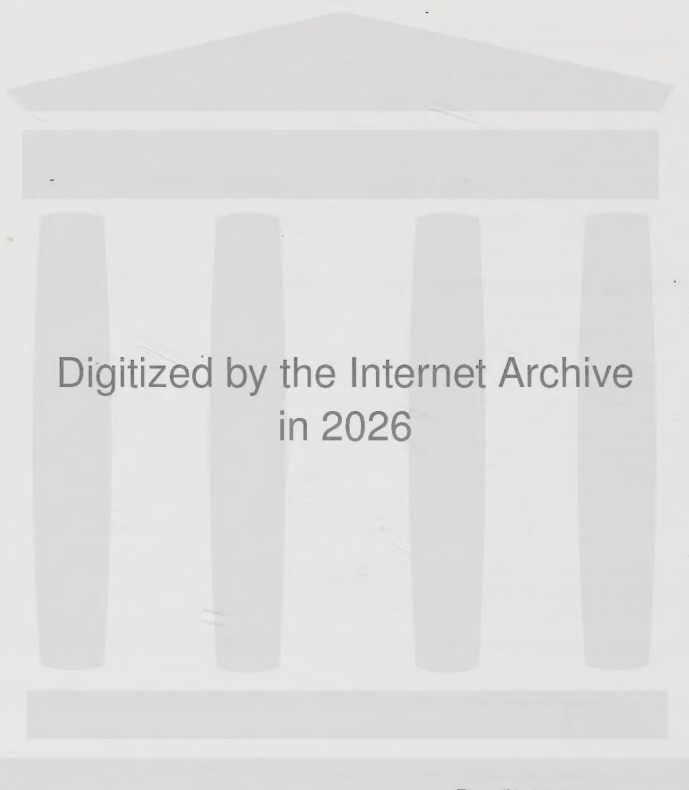
HUMAN VASCULAR α -ADRENOCEPTORS

A study of peripheral arteries and veins in vitro and in vivo

by

Stig Steen

Lund 1984



Digitized by the Internet Archive
in 2026

To Ellen

The present thesis is based on the following articles, which will be referred to in the text by their Roman numerals.

- I Steen, S., Skärby, T., Norgren, L. & Andersson, K.-E.:
Pharmacological characterization of postjunctional α -adrenoceptors in isolated human omental arteries and veins.
Acta Physiol Scand 1984, 120: 109-116.
- II Steen, S., Sjöberg, T., Skärby, T., Norgren, L. & Andersson, K.-E.:
Postjunctional α_1 - and α_2 -adrenoceptors mediating contraction in isolated human groin arteries and veins.
Accepted in Acta Physiol Scand.
- III Steen, S., Sjöberg, T., Skärby, T., Norgren, L. & Andersson, K.-E.:
The postjunctional α -adrenoceptors of the human saphenous vein.
Accepted in Acta Pharmacol et Toxicol.
- IV Steen, S., Castenfors, J. Sjöberg, T., Skärby, T., Andersson, K.-E. & Norgren, L.:
Effects of α -adrenoceptor antagonists on the human saphenous vein in vivo.
In manuscript.
- V Steen, S., Alm, P., Andersson, K.-E., Sjöberg, T., Skärby, T. & Norgren, L.:
Human vascular α -adrenoceptors in the ischemic leg. A study in vitro and in vivo.
In manuscript.

CONTENTS

Introduction.....	5
Aim of the study.....	18
Material.....	19
Methods.....	21
Results.....	32
General discussion.....	50
Clinical applications and future aspects.....	63
General conclusions.....	71
Acknowledgements.....	73
References.....	74
Enclosures I - V.....	89

INTRODUCTION

Basic concepts

Langley (1878) was the first to propose the receptor concept when studying the actions of atropine and pilocarpine on cat salivary secretion (1). Most bioactive agents exert their specific effects in the body by forming a bond, generally reversible, with some cellular constituent. This cellular constituent is called the receptor (2).

Affinity describes the tendency of attachment or binding of a drug to a receptor. Intrinsic activity is related to the ability of the drug to induce an effect after binding. Drugs that possess an affinity for a receptor and also have a measurable intrinsic activity are named agonists. Conversely, drugs which bind to a receptor but have no intrinsic activity (i.e. produce no pharmacological response) are named antagonists. Drugs with high or low affinity can have either high or low intrinsic activity. Partial agonists are drugs that possess agonist activity but which produce smaller responses than other agonists known to act at the same receptor (Fig 1).

Experience shows that a maximal response can be obtained in some tissues with a concentration of a strong agonist well below that required for saturation of the receptor population, i.e. a receptor reserve is obviously present (3). Thus in some tissues, a significant proportion of the receptor pool can be irreversibly inactivated, and yet a maximum response to a strong agonist can

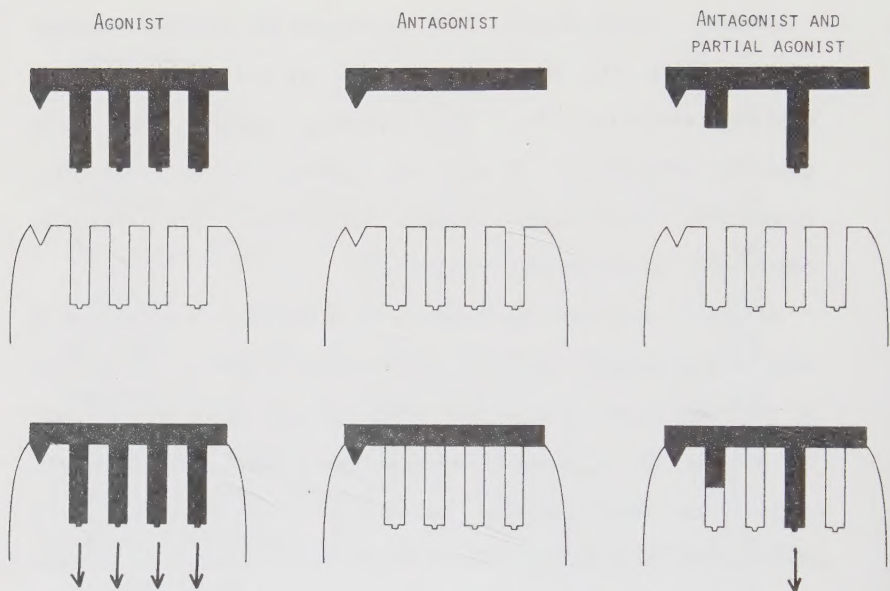


Fig 1. Schematic model showing that a partial agonist, when interacting with a full agonist, in fact is a partial antagonist.

still be attained if higher concentrations of the agonist are administered. Only when the total number of free receptors are reduced below the level required for a maximal response, will depression of the maximum be observed.

Agonists can elicit their effects in often extremely low concentrations. Such agonists have high potency. The interaction of only a few drug molecules with their receptors can induce a massive response in which tremendous numbers of molecules are involved. This requires amplifier systems. The most simple amplifier unit is that in which a molecule of a drug by interaction with its receptor leads to activation of an enzyme molecule which then can convert hundreds of substrate molecules to product molecules, and if these in their turn act as activators of another receptor or enzyme system, a strong amplification is obtained (4) (Fig 2).

The receptors may be located in the outer cell membrane (e.g. adrenergic receptors), in the cytoplasm (e.g. steroid receptors) or in the cell nucleus (e.g. triiodothyronine receptors) (5).

So far, only one type of receptor, i.e. the acetylcholine receptor in the striated muscle cell membrane has been extensively characterized. This receptor can be easily isolated due to radioactively labelled α -bungarotoxin, a neurotoxin which binds irreversibly to the acetylcholine receptor. The toxin can thus serve as a marker in the isolation procedure, giving almost pure receptor substance for analysis (6, 7) (Fig 3). One may suggest that all receptors, like the acetylcholine receptor in striated



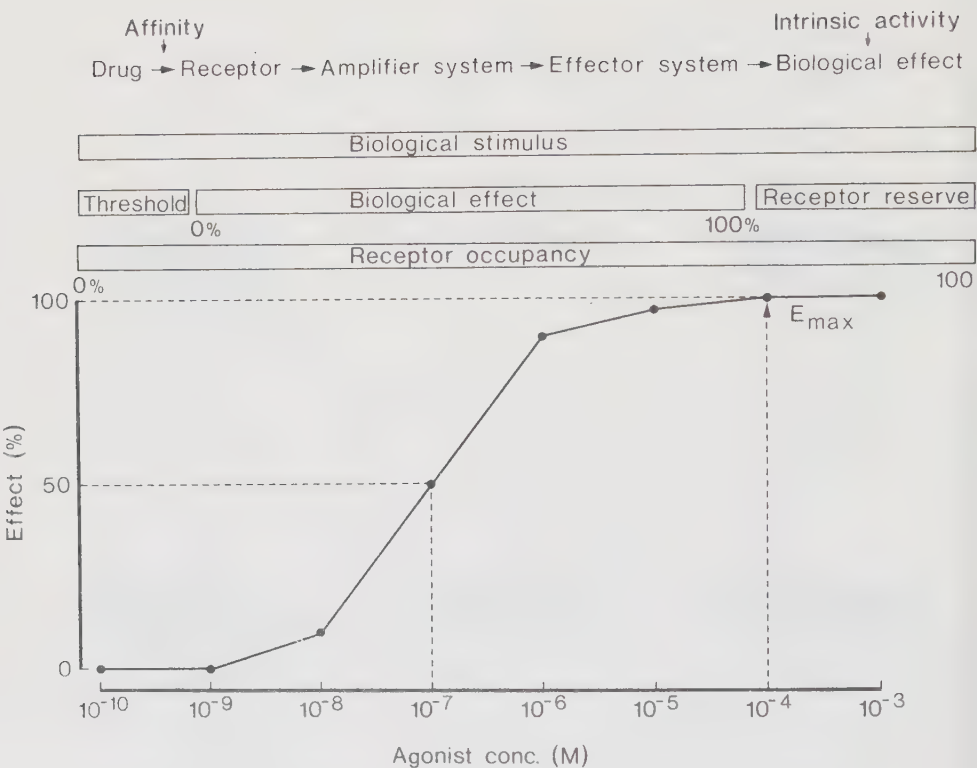


Fig 2. Most bioactive agents elicit their effects in concentrations between 10^{-9} M and 10^{-4} M. $E_{\max}$ is the maximum effect obtained with an agonist. The agonist concentration at which 50 % of maximum effect occurs, gives the EC_{50} -value (10^{-7} M in this example). The negative logarithm of the EC_{50} -value is the pEC_{50} -value ($-(-7)=7$ in this example). In certain tissues, a significant fraction of the receptors may have to be occupied before an effect is observed; a typical dose-response curve as shown in the present example, is then obtained only when the stimulus increases beyond a certain threshold. Since $E_{\max}$ can be a physical limitation of the tissue, it may occur at some degree of receptor occupancy less than saturation, i.e. a receptor reserve is present.

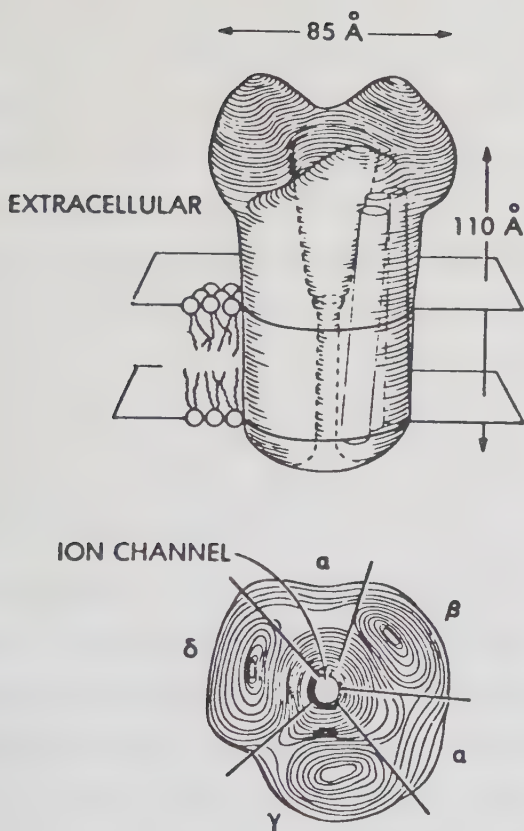


Fig 3. Schematic three dimensional model of the acetylcholine receptor in the striated muscle cell membrane. The protein topography was deduced from electron-microscopy and X-ray diffraction (6).

muscle or like enzymes, are proteins. When stimulated conformational changes in the protein molecule probably trigger the cascade of events leading to the final response.

Adrenergic receptors (Adrenoceptors)

In 1905 Langley postulated that adrenaline produced its effects by combining with "receptive substances" in the effector organs (8). He also suggested the occurrence of different types of receptive substances within the same tissue, and that this determined whether the organ would be stimulated or inhibited by a drug. Dale (1906) found that ergot alkaloids inhibited most of the excitatory, but not the inhibitory effects of adrenaline. Until 1948 the adrenoceptors were considered to be either excitatory or inhibitory. Experiments on dogs, cats, rats and rabbits performed by Ahlquist in 1948 indicated that, although there were two classes of adrenoceptors, they could not be classified simply as excitatory or inhibitory since each kind of receptor could have either action depending upon where it was found (9). The brief evidence for these conclusions was that a series of six sympathomimetic amines had one order of potency - 1 (noradrenaline), 2, 3 (adrenaline), 4, 5, 6 (isoprenaline) - on the following functions: vasoconstriction, excitation of the uterus and ureters, dilatation of the pupil and inhibition of the gut. In contrast, the same series of amines had an entirely different order of potency - 2, 4, 6 (isoprenaline), 5, 3 (adrenaline), 1 (noradrenaline) - on the following functions: vaso-

dilatation, inhibition of the uterus and myocardial stimulation. The first kind of receptor (NA > A > Iso) was called the α -adrenotropic receptor and the second kind (Iso > A > NA) the β -adrenotropic receptor. This classic study of Ahlquist has had widespread practical ramifications for the development of new therapeutic agents (10).

In 1967 Lands et al (11) found that the order of potency of several β -receptor agonists could be classified into two main groups when tested on several isolated organs. This made him suggest the existence of β_1 - and β_2 -receptors.

α - Adrenoceptors (α -receptors)

After Ahlquist's pioneer work it soon became well established that noradrenaline (NA) is the neurotransmitter released at adrenergic nerve endings in the peripheral nervous system (12). In transmitter release studies carried out with ^3H -NA it was shown that α -receptor blockers like phentolamine increased the overflow of ^3H -NA elicited by nerve stimulation 10-20-fold, whereas α -receptor agonists inhibited the transmitter overflow (13). These and other results led to the hypothesis of a presynaptic regulation of NA release through a negative feedback mechanism mediated by α -receptors. When the potency of the α -receptor blocker phenoxybenzamine in blocking the pre- and postsynaptic α -receptors was tested in the perfused cat spleen (i.e. measuring perfusion pressure changes, keeping the perfusion rate constant) it was found that a significant reduction in responses to nerve

stimulation was obtained in the presence of low concentrations of phenoxybenzamine, although transmitter release was not increased under these experimental conditions; the difference between the concentration of phenoxybenzamine required for blockade of the postsynaptic α -receptors and that necessary to enhance transmitter release during nerve stimulation was 30-fold (13). Due to these and other results Langer (1974) suggested that the postsynaptic α -receptors which mediate the response of the effector organ should be referred to as α_1 , while the presynaptic α -receptors which regulate transmitter release should be named α_2 (13). This anatomical subclassification of α -receptors was questioned by Berthelsen and Pettinger (1977) who pointed out that α -receptors with α_2 -characteristics also could be found postsynaptically, e.g. the α_2 -receptors inhibitory to MSH-induced darkening of the frog skin (14). Further research into this field has led to a classification of α -receptor subtypes exclusively on pharmacological grounds (15 - 18). The subclassification of α -receptors into α_1 - and α_2 -types is based on differences in relative potencies for a range of α -receptor selective agonist and antagonist drugs. The term α_1 -receptor is used for a receptor that is preferentially stimulated by phenylephrine and blocked by prazosin, whereas the term α_2 -receptor is used for a receptor that is preferentially stimulated by clonidine and blocked by rauwolscine or yohimbine.

Vascular α -receptors

One of the important pathways for the regulation of blood pressure by the central nervous system is control of the total peripheral resistance. This control is mediated by moderation of the vascular smooth muscle tone via the sympathetic division of the autonomic nervous system. The final chemical transmitter released by these nerves onto the receptors of vascular smooth muscle is noradrenaline. It has been demonstrated that these postjunctional adrenoceptors are of two main types, α_1 , mediating contraction and β , mediating relaxation, although the α -receptors seem to predominate in most vascular beds (19). The presynaptic autoinhibitory α_2 -receptors at the vascular neuro-effector junction have been studied both in vitro and in vivo in animals and man and seem to be similar to the α_2 -receptors found presynaptically in other tissues (20 - 24). However, the nature of the postjunctional α -receptors mediating vasoconstriction is still a controversial matter.

The postjunctional α -receptors in animal vascular preparations

Experiments on perfused hindlimb of rat (25), rabbit (26), cat (27) and dog (27, 28, 29) indicate that the resistance vessels in these tissues are endowed with contraction mediating postjunctional α -receptors of both α_1 -and α_2 -subtype. Table I shows the suggested subtypes of postjunctional α -receptors as found in vitro in isolated vascular preparations from different regions in different animal species.

Table I

Suggested subtypes of contraction mediating postjunctional α -receptors as found in several in vitro experiments using isolated vascular preparations from different regions and species

Predominantly α_1 -receptors postjunctionally

Rat middle cerebral artery (30)

Aorta from guinea pig, rabbit, cat, dog and hamster (31)

Rabbit pulmonary artery (32)

Dog femoral artery (33)

Rat and rabbit portal vein (34, 35)

Predominantly α_2 -receptors postjunctionally

Rat aorta (36)

Cat middle cerebral artery (37)

Dog basilar artery (38)

Mixed population of α_1 - and α_2 -receptors postjunctionally

Cat lingual artery (37)

Dog saphenous vein (33, 39, 40)

None contraction-mediating α -receptors postjunctionally

Rat basilar artery (30)

Due to lack of better technique most in vitro experiments are hampered by being performed on large caliber vessels which may be less interesting from a physiological point of view. One may suggest that small caliber muscular vessels are more endowed with receptors than large elastic vessels. The consistent demonstration of contraction mediating α -receptors in the above mentioned hindlimb perfusion experiments, but not in the in vitro experiments, may indicate that the arteries used in the in vitro experiments have been too large. But still, the animal experiments indicate some important points concerning postjunctional α -receptor subtypes, namely that there are species and regional variations as well as differences between arteries and veins (Table I).

The postjunctional α -receptors in human vascular preparations

The literature of systematic studies of postjunctional α -receptors in human vascular preparations is scarce and certain criticism may be raised against it. Stevens and Moulds (41) studied spiral strips of digital arteries and metacarpal veins obtained at autopsy between 8 - 30 h after death. Glusa and Markwardt (42) investigated helically cut strips of femoral veins (obtained up to 8 h after death) and femoral arteries (obtained up to 15 h after death) from autopsied patients.

These studies may be less relevant in the physiological situation since they used post mortem vessels from not further

defined patients (see V in the present study). In Glusa and Markwardt's study the veins and arteries were obtained at different times after death. Holmberg et al (43) have shown that innervated human vessel preparations increased their sensitivity and maximal response to noradrenaline after storage, and they suggested that this could be explained by denervation supersensitivity and receptor upregulation. If therefore a characterization and comparison of postjunctional α -receptors between different vessel preparations shall be properly done, the studies ought to be performed at the same postdenervation time, and this time should be as short as possible.

Methods for studying vascular α -receptors

Most data about vascular α -receptors have been obtained in functional studies by recording the mechanical response of different α -receptor selective drugs on the vascular smooth muscle contraction.

Electrical nerve stimulation is used to elicit release of transmitter for "physiological" stimulation of the receptors. Transmitter release studies are done by preloading vascular preparations with e.g. ^3H -NA for subsequently electrical stimulation and registration of the ^3H -efflux. By manipulating the transmitter release with α -receptor subtype selective agonists and antagonists, much information about the presynaptic α_2 -receptors has been obtained. However, the only approach to study

α -receptors directly is with radioligand binding technique (44). Radioactively labelled selective α -receptor antagonists and agonists are used. Such studies give information about the receptor number and affinity but cannot discriminate prejunctional from postjunctional receptors, and give no information about the functional significance of the receptor populations found; e.g. estrogen treatment of rabbits caused a dramatic increase in the number of α_2 -receptors in the uterus with no significant change in the number of α_1 -receptors; functional studies, however, demonstrated that myometrical contraction was mediated exclusively by α_1 -receptors (45). Thus functional studies must always be done to confirm the significance of different receptors demonstrated by radioligand binding techniques.

The advantage with functional in vitro studies is that the experimental conditions can be kept under well defined control, without presence of unknown blood borne substances or nervous reflex mechanisms. But results obtained in vitro have to be interpreted with caution regarding their potential physiological relevance; e.g. Schümann and Lues (46) concluded that the post-junctional α_2 -receptors of the rabbit saphenous vein required the blood borne substance angiotensin for their expression in vitro. Functional receptor studies in vivo may be impossible to interpret because of unknown nervous or hormonal compensation mechanisms. Thus, a combination of functional in vitro and in vivo investigations seems to be the only safe pathway to follow.

The need for further studies regarding vascular α -receptors

Since differences between species are known to occur (Table 1) studies should be performed in man.

Vessels obtained from living persons ought to be used, and what is found valid in healthy subjects may not be valid in patients. Arteries and veins must be considered as different structures, and nothing found to be valid in arteries must be extrapolated to veins. Different regions have to be investigated since animal studies have shown great regional variation. Furthermore, vessels of different calibers must be investigated, suspecting that e.g. "distributing arteries" may have other receptors than "resistance arterioles" or that thickwalled subcutaneous veins may behave differently from fragile muscle - or visceral veins. In vitro results have to be confirmed in vivo before being considered biologically valid.

AIM OF THE PRESENT STUDY

The aim of the present study was to investigate α -adrenoceptors in human peripheral blood vessels

- 1) from different regions in healthy adults;
- 2) from ischemic legs in patients with severe atherosclerosis;
and
- 3) to compare arteries and veins;
- 4) to compare in vitro and in vivo findings.

Choice of regions

The aim was to investigate the vascular α -receptors in a visceral and a somatic region of surgical importance.

Mesenteric vessels are of obvious surgical importance and easy to obtain during laparotomy and represent therefore visceral vessels in the present study.

Groin vessels are also easily obtained during varicose vein surgery. Due to the development of microsurgery, the blood vessels of the groin region have come into great interest. To correct tissue defects of trauma patients, a free flap transfer involving microvascular anastomoses is often used, and this free flap is usually taken from the groin, raised on the groin vessels; in this way an enormous area of skin can be transplanted freely to any area of the body (47, 48). Blockade of α -receptors has been recommended to achieve the best possible blood flow through such free flaps (49). Therefore, information about the α -receptors in groin vessels may be of particular interest.

The long saphenous vein is a most important vessel in cardiovascular surgery because it is the graft of choice in coronary and femoropopliteal by-passes as well as in peripheral vascular trauma surgery. It is well known among surgeons that traumatic dissection of the vein elicits a pronounced contraction of the vessel making it difficult to handle. Thus, it is of obvious clinical interest to obtain information on the factors regulating

contraction and relaxation in this vessel which also represents a large caliber vein contra the small caliber groin veins.

I:

Branches of the gastroepiploic vessels were taken from fifteen patients (8 women and 7 men, aged 18 - 77, mean 54 years) undergoing laparotomy because of gall stones (8), peptic ulcer (3), hiatic hernia (1), appendicitis (1) and abdominal aortic aneurysm (2). Only patients free from "chronic" drug treatment were chosen.

II:

Twenty healthy patients (12 women and 8 men, aged 23 - 71, mean 47 years) operated on because of varicose veins were vessel donors. A segment of the superficial external pudendal artery and small groin vein segments were used.

III:

Seventeen healthy patients (11 women and 6 men, aged 31 - 65, mean 48 years) operated on because of varicose veins were vessel donors. The long saphenous vein at the medial malleolus (if macroscopically normal) was used in the experiments.

IV:

Six healthy male subjects (aged 25 - 35 years) participated in the study. Only subjects with a well visible long saphenous vein at the medial malleolus were chosen (none of them had varicose veins).

V:

Sixteen patients (4 women and 12 men aged 42 - 92, mean 67 years) undergoing femoro-popliteal bypass procedure because of severe ischemia due to atherosclerosis participated in the study. From 8 of the patients small branches from the femoral and posterior tibial vessels within the muscles were secured for in vitro studies.

METHODS

Radioligand binding - and nerve stimulation studies on human vascular preparations are under development in our laboratory and only pilot studies have so far been done with these techniques. Radioligand binding studies on human blood vessel are hampered by the difficulty of obtaining a sufficient amount of tissue. Release-studies, giving information about presynaptic receptors, were not done because little controversy exists at present about the presynaptic α -receptors.

Because of access to a newly developed (50) sensitive myograph

for recording mechanical activity in isolated small blood vessels (calibres down to 100 μm), the basal characterization of post-junctional α -receptors was performed using this myograph.

A device for measuring changes in size of subcutaneous veins (51) was especially useful in studying the in vitro/in vivo connection because the device could be placed over the long saphenous vein at the medial malleolus and thus give in vivo information from exactly the same location as used in vitro. This is important since Thulesius (52) has suggested that nor-adrenaline is more potent in the distal than in the proximal parts of the long saphenous vein. There is no evidence for an abnormality in the smooth muscle in the veins from varicose patients (52, 53). This would otherwise have disturbed the results obtained on veins from such patients, although these vessels were macroscopically normal.

Electromagnetic blood flow measurements have been a per-operative routine during by-pass surgery in our department for many years and it was therefore quite natural to use it in the present study.

At present there is limited experience with laser doppler technique in measuring skin blood flow variations. The results obtained with this technique (V) are interpreted according to Tenland (54)

The in vivo data obtained in study V are "peak" responses, which may be misleading if pre- and postjunctional effects "peak" at different times (55). It would have been better to administer

all drugs in continuous infusions and record the steady state responses, but that would have prolonged the operations too much. Moreover, the primary aim of the study was to obtain information about the α -receptor mediated contraction and relaxation capacity of vessels in severe ischemic legs. The doses of the drugs used in this respect would have given side effects if given in continuous infusions to a steady state plasma level.

Recording of mechanical activity in isolated small arteries and veins in vitro. (I, II, III, V)

All vessels extirpated were immediately placed in chilled Krebs solution and transported to the laboratory for further dissection under an operation microscope. Care was taken to avoid stretching or other types of injury to the vessels. From each artery or vein approximately 1 mm long segments were prepared, some of which were immediately mounted in temperature-controlled (37°C) organ baths filled with Krebs solution (Fig 4) whereas the remainder was kept in Krebs solution (4°C) for later use (within 24 h). Cocaine (10^{-6}M) for blockade of neuronal uptake and propranolol (10^{-7}M) to block β -adrenoceptors were present in the baths which were continuously bubbled with a gas mixture of 5% CO_2 and 95% O_2 giving a pH of approximately 7.4. The vessel segments were suspended between two L-shaped metal holders (diameter 200 μm for most vessels, 50 - 100 μm for the smallest vessels). One of the metal holders was attached to a Grass FT03 transducer connected to a Grass polygraph for continuous

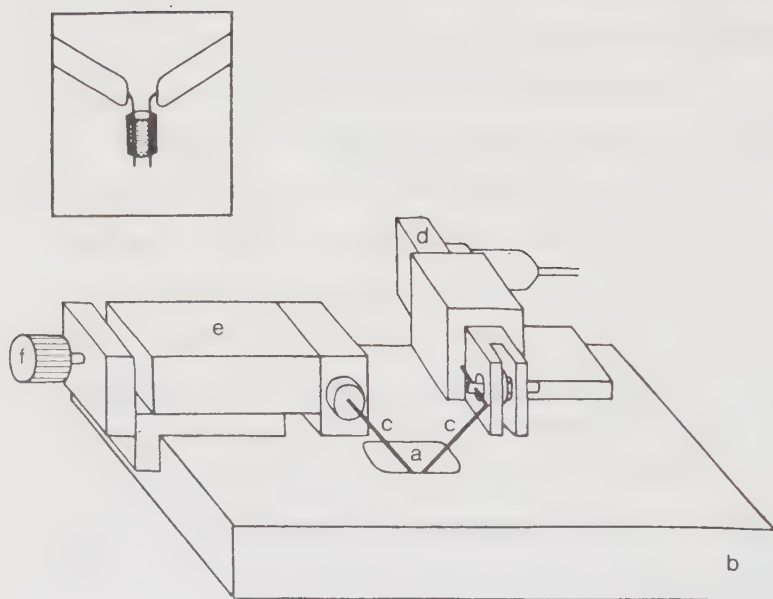


Fig. 4. Schematic drawing of the myograph.

- a. Organbath, 2.5 or 5 ml
- b. Myograph block
- c. Metal holders
- d. Force-displacement transducer
- e. Displacement unit
- f. Micrometer screw to manipulate e

The gas mixture was conducted through a small tubing, entering at the back of the Perspex block, to the bottom of the organbath. Heated water, delivered by a thermostatically controlled pump unit, was continuously circulating around the muscle bath inside the myograph block.

recording of isometric tension. The position of the other holder could be changed by means of a movable unit allowing fine adjustments of vessel wall tension. The K^+ (124mM)-induced contraction was used as an internal standard in each vessel segment tested, and each agonist response was expressed as a percentage of the K^+ -induced contraction. In separate experiments it was secured that pretreatment with phentolamine ($10^{-6}M$) reduced the K^+ -induced contraction by less than 10%. It was also shown in preliminary experiments that an increase in cocaine or propranolol concentrations beyond those used in this study did not potentiate the NA response.

Histological examination(V)

Before and after the experiments in the organ baths, vessel segments were fixed in 10% formol, dehydrated, embedded in paraffin, sectioned, stained and histopathologically evaluated.

Recording of changes in size of the saphenous vein in vivo (IV)

Changes in size of the long saphenous vein at the medial malleolus was measured using a device described by Castenfors and Felding (51). The vein was illuminated from one side with 940 nm infrared light through a 0.2 mm vertical slit. On the other side of the vein variations in light intensity induced by changes in diameter of the vein were measured with a matched phototransistor (Fig 5). The subjects were placed in supine position in a warm quiet room ($28 - 30^{\circ}C$) to minimize endogenous

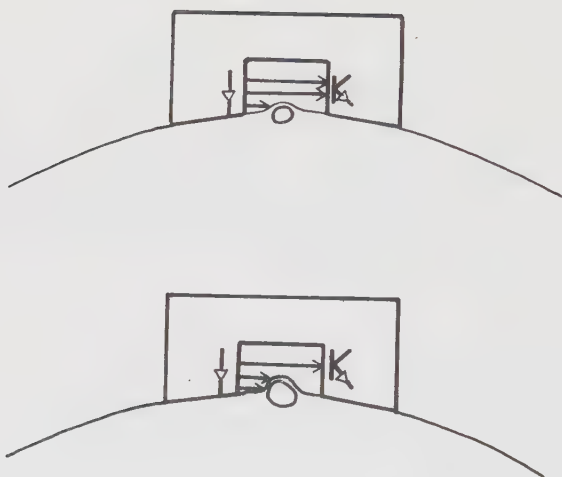


Fig 5. The device used to register changes in venous diameter. ∇ symbolizes a photodiode giving 940 nm infrared light through a 0.2 mm vertical slit. ∇ symbolizes a phototransistor measuring variations in light intensity.

sympathetic tone. The legs were elevated just above the heart level to achieve "collapsed" saphenous veins (zero level). A sphygmomanometer cuff was applied just below the knee and an occlusion pressure of 40 mmHg was applied, inducing a stable expansion of the saphenous vein. Through an intravenous needle, the point of which was placed 3 - 4 mm distal to the measuring device, infusion of 0.9% NaCl alone or with addition of different drugs was given with a constant infusion rate of 0.3 ml/min.

Electromagnetic blood flow measurements (V)

The registrations were done as the first phase of the operation of patients with severe leg ischemia planned for a femoro-popliteal by-pass. A Cliniflow electromagnetic blood flowmeter (Carolina Medical Electronics, Model 601 D) was used. A well fitting flow probe was placed on the deep femoral artery close to the bifurcation (the superficial femoral artery was occluded because of atherosclerosis) (Fig 6). The drugs were given via a Venflon cannula into the common femoral artery.

Measurement of skin blood flow variations with laser doppler technique (V)

The registrations were done simultaneously with the electromagnetic blood flow measurements. A Periflux Laser Doppler Flowmeter PF1c (Perimed KB) was used. The measuring probe was placed on the skin of the big toe or foot sole; if gangrene of the foot was present the probe was placed on the skin just

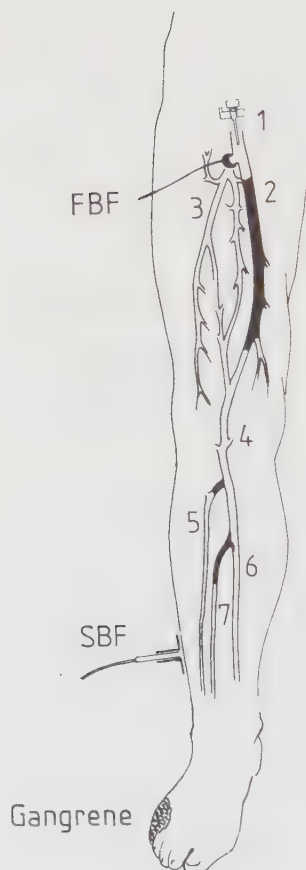


Fig 6. Measurements of femoral artery blood flow (FBF) and of variations in skin blood flow (SBF) with help of an electromagnetic blood flowmeter and a laser doppler flowmeter, respectively.

- 1 = common femoral artery (punctured by a Venflon catheter)
- 2 = superficial femoral artery (occluded due to atherosclerosis)
- 3 = deep femoral artery
- 4 = popliteal artery
- 5 = anterior tibial artery
- 6 = posterior tibial artery
- 7 = peroneal (fibular) artery

proximal of the lateral malleolus (Fig 6). The output signal from the flowmeter registered in volt, was linearly related to the flux of red cells, i.e. the number of cells times their velocity. The laser light penetrated about 1 mm down in the outermost layer of the skin, and the diffuse scattering of light in tissue made the recorded flow value virtually independent of flow direction (54, 56, 57).

Pharmacological tools

The α -receptor subtype selective agonists and antagonists used in this study are shown in Table 2.

Table II

The α -receptor subtype selective drugs used in the present study.

Agonists:

Antagonists:

	α_1	
Phenylephrine	↑	Prazosin
Cirazoline		
ST-587		
Noradrenaline	$\alpha_1 = \alpha_2$	Phentolamine
Oxymetazoline		
BHT-933		
Guanfacine		
Naphazoline		
BHT-920		Yohimbine
Clonidine	↓	Rauwolscine (α -yohimbine)
	α_2	

Calculations

All concentration-response curves were plotted graphically and $E_{\max}$, i.e. the maximum contraction obtained with an agonist, and EC_{50} , i.e. the agonist concentration at which 50% of maximum effect occurs, were calculated. pEC_{50} (or pD_2) was defined as the negative logarithm of the EC_{50} -value. pA_2 , i.e. the negative logarithm of the antagonist concentration that displaced the agonist concentration-response curves towards higher concentrations with a factor of 2, was determined according to the method of Arunlakshana and Schild (Schild plot) (58, 59, 60). The concentration ratio (CR) was calculated as the ratio between the EC_{50} -values in presence and in absence of antagonist.

Paired or unpaired Student's t-test was used in the statistical analysis of the results. A probability level lower than 0.05 was accepted as significant. The regressions of the Schild plots, based on linear least square fit, were performed on a calculator (Hewlett Packard 41 CV) using a program kindly supplied by Dr Claus Rerup, Department of Pharmacology, University of Lund. The results are given as mean with 95% confidence intervals or as mean $\pm$ SEM.

RESULTS

I. The postjunctional α -receptors in human omental arteries and veins

Omental arteries

Both prazosin (pA_2 9.48) and rauwolscine (pA_2 7.19) displaced the NA concentration-response curve towards higher concentrations without reduction of maximum (Fig 7). Neither of the α_2 -receptor agonists used (i.e. clonidine and oxymetazoline) had any consistent contractile effects. Phenylephrine (pEC_{50} 5.62) had a lower potency than NA (pEC_{50} 6.07), but a similar intrinsic activity.

Omental veins

Both prazosin (pA_2 9.72) and rauwolscine (pA_2 8.11) displaced the NA concentration-response curve towards higher concentrations, but also significantly depressed maximum (Fig 7). Clonidine and oxymetazoline contracted veins from 3 out of 7 and 4 out of 6 patients, respectively. Their pEC_{50} -values were similar to that of NA, but their intrinsic activities were significantly lower. NA (pEC_{50} 6.93) was more potent than phenylephrine (pEC_{50} 5.65) but there was no significant difference in intrinsic activity.

NA was significantly more potent in the veins than in the arteries whereas the intrinsic activity was similar in the two

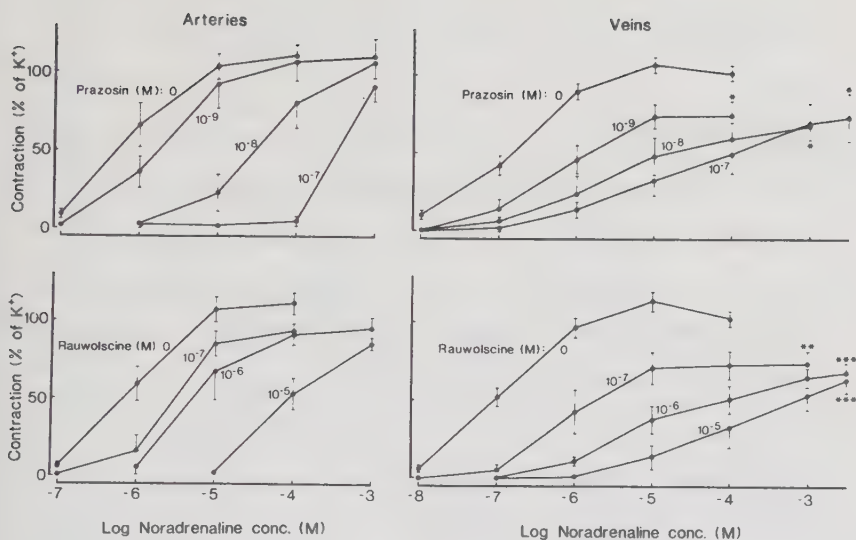


Fig 7. Concentration-response curves for NA in human omental vessels in the absence and presence of increasing concentrations of prazosin (upper panel) and rauwolscine (lower panel). Arteries are shown to the left, and veins to the right. The contractile responses are expressed as percentage of K^+ (124 mM)-induced contraction. Significant reductions in the maximum response to NA are denoted: * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$). Each point in the curves indicates mean $\pm$ standard error of results obtained from 6 patients.

preparations. (Fig 8). Rauwolscine was also significantly more potent in the veins than in the arteries, whereas the potency for phenylephrine and prazosin was almost similar in arteries and veins.

The results from this study suggest that in human omental arteries, the postjunctional α -receptors are mainly of the α_1 - type, even if a small population of α_2 -receptors cannot be excluded. In omental veins, there seems to be a functionally important population of postjunctional α_2 -receptors occurring together with a population of α_1 -receptors.

II. The postjunctional α -receptors in human groin arteries and veins.

Groin arteries

Prazosin caused a concentration-dependent displacement of the noradrenaline concentration-response curve without reduction of maximum (pA_2 9.86) (Fig 9). Rauwolscine 10^{-8} M caused a rightward shift of the NA concentration-response curve without reduction of maximum, but 10^{-7} M and 10^{-6} M caused little or no further shift and no pA_2 -value could therefore be calculated (Fig 9).

Groin veins

Prazosin 10^{-9} M significantly displaced the NA concentration-response curve, but 10^{-8} M and 10^{-7} M caused little or no further

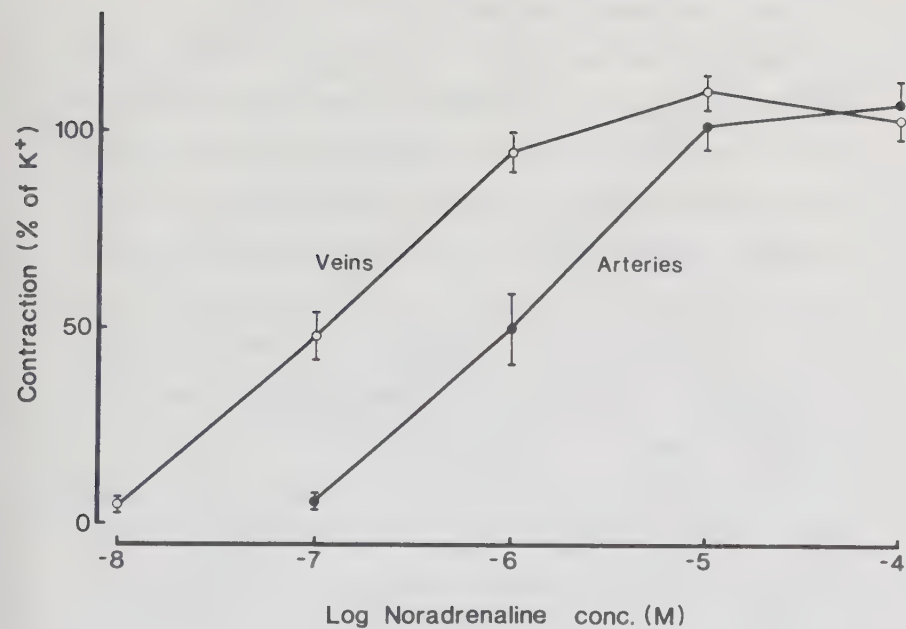


Fig 8. Concentration-response curves for NA in human omental arteries (●) and veins (○) expressed as percentage of the K⁺ (124 mM)-induced contraction. Each point is the mean $\pm$ standard error of segments from 8 patients.

shift (Fig. 9). Rauwolscine caused a concentration-dependent right-ward shift of the NA concentration-response curve without significant depression of maximum (pA_2 9.03) (Fig 9).

NA was more potent in the veins (pEC_{50} 7.00) than in the arteries (pEC_{50} 6.27), but displayed a higher intrinsic activity in the arteries than in the veins. Phenylephrine was more potent in the arteries (pEC_{50} 6.41) than in the veins (pEC_{50} 5.34) and had higher intrinsic activity in the arteries. Clonidine did not elicit contractions in the arteries but contracted all the vein segments tested (pEC_{50} 6.24). The potency and intrinsic activity of oxymetazoline did not differ significantly between the arteries and veins.

The main conclusion from this study is that in human groin vessels, there is a functional predominance of α_1 -receptors in the arteries and of α_2 -receptors in the veins.

III. The postjunctional α -receptors of the human saphenous vein

Rauwolscine as well as yohimbine caused concentration dependent shifts of the NA concentration-response curves and the Schild plot resulted in pA_2 -values of 9.00 and 8.27 respectively. The slope for rauwolscine (0.68) differed significantly ($p < 0.02$) from 1.

Prazosin 10^{-9} M appeared to shift the NA concentration-response curve to the right. However, the shift was not

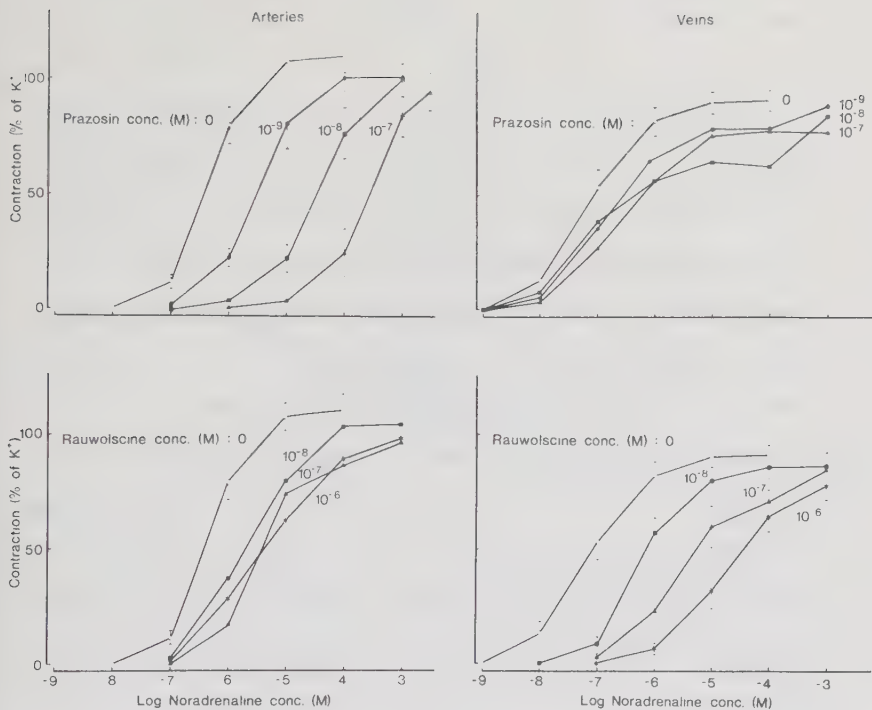


Fig 9. Concentration-response curves for NA in human groin vessels in the absence and presence of increasing concentrations of prazosin (upper panel) and rauwolfscine (lower panel). Arteries are shown to the left, and veins to the right. The contractile responses are expressed as percentage of the K^+ (124 mM)-induced contraction. Each point in the curves indicates mean $\pm$ standard error of results obtained from 6 patients.

significant, and 10 and 100 times higher prazosin concentrations caused little or no further shift. No pA_2 -value could therefore be calculated. No concentration of any antagonist used significantly attenuated maximum, and the concentration-response curves for the NA-controls showed no significant tachyphylaxis.

The relation between potency and intrinsic activity for the α -receptor agonists used is shown in Fig 10. Clonidine had the same potency as NA, but was 52 times more potent than phenylephrine. Phenylephrine and guanfacine had intrinsic activities that did not differ significantly from that of NA, whereas the maximum values of the other agonists were significantly lower.

The main conclusion from this study is that the human saphenous vein is endowed with α_2 -receptors postjunctionally although the existence of a small population of α_1 -receptors cannot be excluded.

IV. Effects of α -receptor antagonists on the human saphenous vein in vivo

Fig 11 shows the local effect of intravenous infusion (0.3 ml/min) of prazosin and yohimbine on the congested (40 mmHg) saphenous vein, which was precontracted by simultaneous local NA-infusion (10^{-7} M NA). Prazosin 10^{-8} M (concentration in syringe) caused a significant inhibition of the NA-contraction in the veins of all subjects. In subjects 3 and 4 no NA-

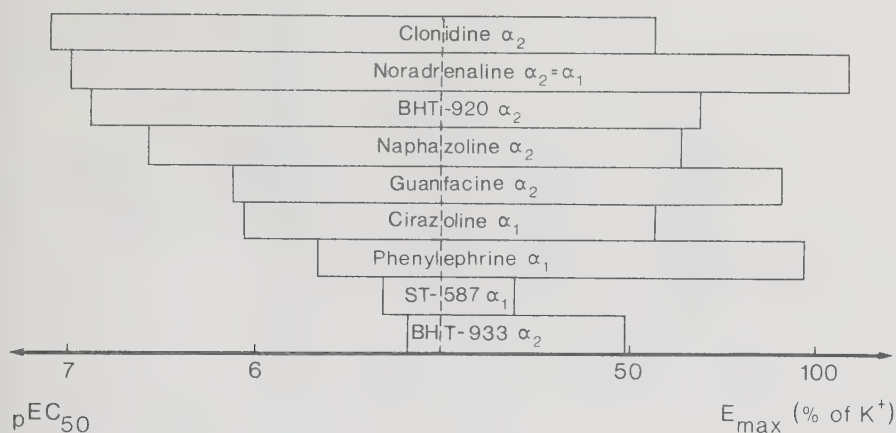


Fig 10. The relation between potency and intrinsic activity for 9 α -adrenoceptor selective agonists, expressed as pEC_{50} and E_{max} , respectively. The effects of all drugs were examined on human saphenous vein. $n = 6$ except for NA in which $n = 14$.

contraction was elicited in presence of 10^{-8} M prazosin. Prazosin 10^{-7} M caused no further blockade of the NA-contraction in subjects 2,5 and 6.

Yohimbine caused a dose-dependent blockade of the NA-contraction and was not quite as potent as prazosin except in subject 1 where yohimbine was especially potent (Fig 11).

This study suggests that the human saphenous vein is endowed with functionally important populations of both α_2 - and α_1 - receptors.

V. Vascular α -receptors in the ischemic leg

Functional studies in vitro

In the arteries great intra- and interindividual variations in the responses to potassium, NA, phenylephrine and clonidine were seen (Table III). Furthermore, several arteries were not able to contract at all, and a great tendency of tachyphylaxis was seen in the arteries, making the interpretation of antagonist experiments impossible.

On the other hand, in the veins well reproducible contractions were obtained. The order of potency for the agonists tested in the veins were: clonidine = NA > phenylephrine (Table III). Fig 12 shows an antagonist experiment using a small fragile vein segment (diam 1 mm) taken within the soleus muscle from a 77 year old woman (patient VIII in Table III) with severe ischemia due to atherosclerosis and diabetes mellitus; as seen from Table III,

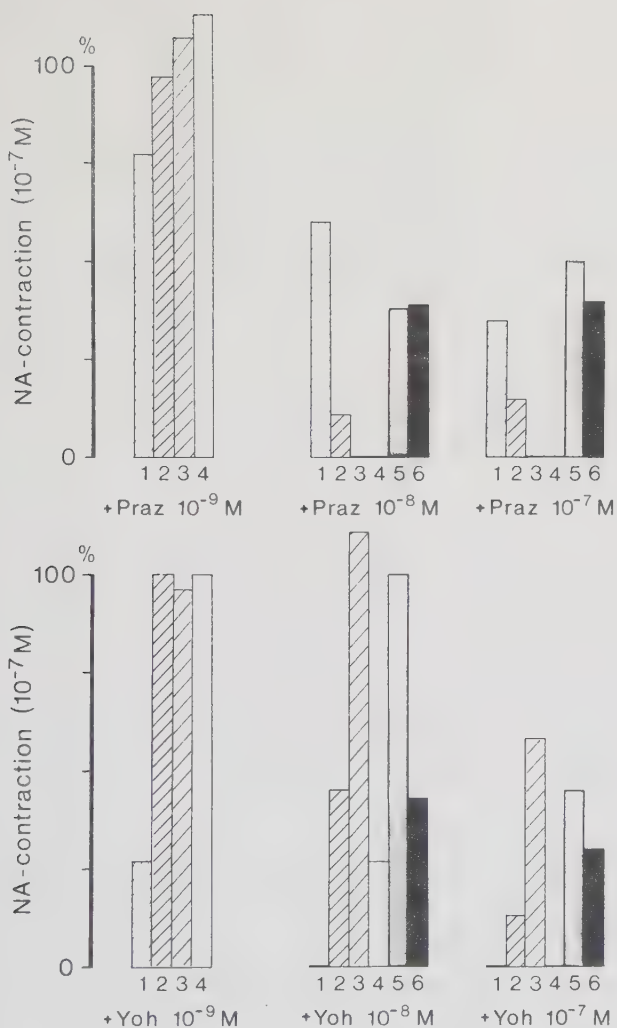


Fig 11. The contraction induced by 10^{-6.8} (10⁻⁷) M noradrenaline (NA) in veins from six subjects (1 - 6) in presence of prazosin (Praz, upper panel) and yohimbine (Yoh, lower panel). All drugs were given with a continuous infusion rate of 0.3 ml/min. The contraction induced by NA in absence of antagonists was defined as 100%.

Table III Effects of α -adrenoceptor agonists on isolated crural arteries and veins obtained from patients with severe leg ischemia
The contractile responses are expressed as percentage of the K^+ (124 mM) -induced contraction.

	K^+ (mM)	Noradrenaline (α_1 & α_2) E_{max} (% of K^+) pEC_{50}	K^+ (mM)	Phenylephrine (α_1) E_{max} (% of K^+) pEC_{50}	K^+ (mM)	(Clonidine (α_2) E_{max} pEC ₅₀ (% of K^+))
I	0	0				
II	0	0	0	0	0	0
III	5	169	29	99	52	0
IV	0	0	2	6	12	0
	12	0				
	6	32				
V	0	20	17	0	22	0
	17	0				
	12	0				
VI	9	60				
VII	31	34	17	33	22	14
VIII	0	0				6.54
I	35	114	40	100	75	68
II	37	53	26	70	26	14
III	17	119				5.62
VI	12	91	17	91	29	54
						6.49
VIII	17	124				
	23	109				
	19	135				

ARTERIES

VEINS

strong contractions could be elicited and NA was especially potent (pEC_{50} 7.38). A small artery segment (diam 1 mm) taken for comparison from the same region in the same patient was unable to contract upon exposure to potassium (124 mM) or NA. As seen in Fig 12 rauwolscine, but not prazosin, caused a concentration dependent rightward shift of the NA concentration - response curve.

Histopathological findings

The vessel segments, representing small muscular arteries from ischemic legs, displayed small to moderate changes. Generally, the intima was focally thickened due to increased amounts of subendothelial fibrous tissue in which a few cells with a foamy cytoplasm were found. Further, the internal elastic membrane had focally disappeared whereas in other areas there were only scattered remnants. The media showed increased fibrosis with a reduced number of smooth muscle cells, which were often hypertrophic and had nuclei which were slightly pleomorphic in shape and had a hyperchromic staining intensity giving an impression of slight degeneration. The morphologic pictures were compatible with slight to moderate atherosclerosis. In the vessel segments taken from small veins from the same patients no overt histopathological findings were noticed. Fig 13 b gives a typical example, showing a small muscular artery taken from patient I in Table III. This artery was unable to contract on exposure to potassium (124 mM) or NA. For comparison is shown a small muscular artery taken from a 53 year old healthy woman (Fig 13 a).

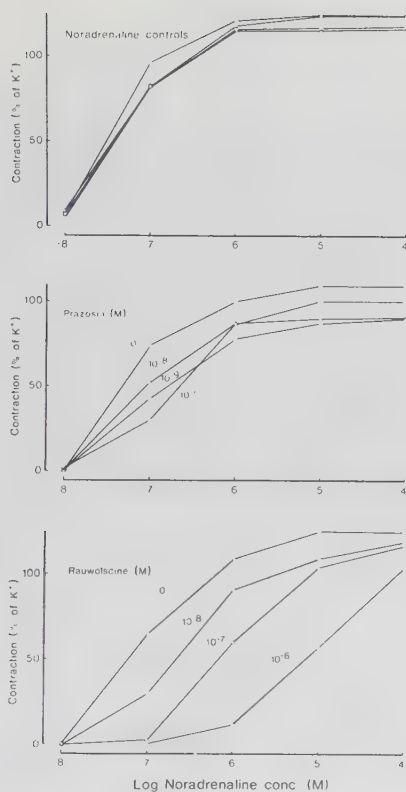
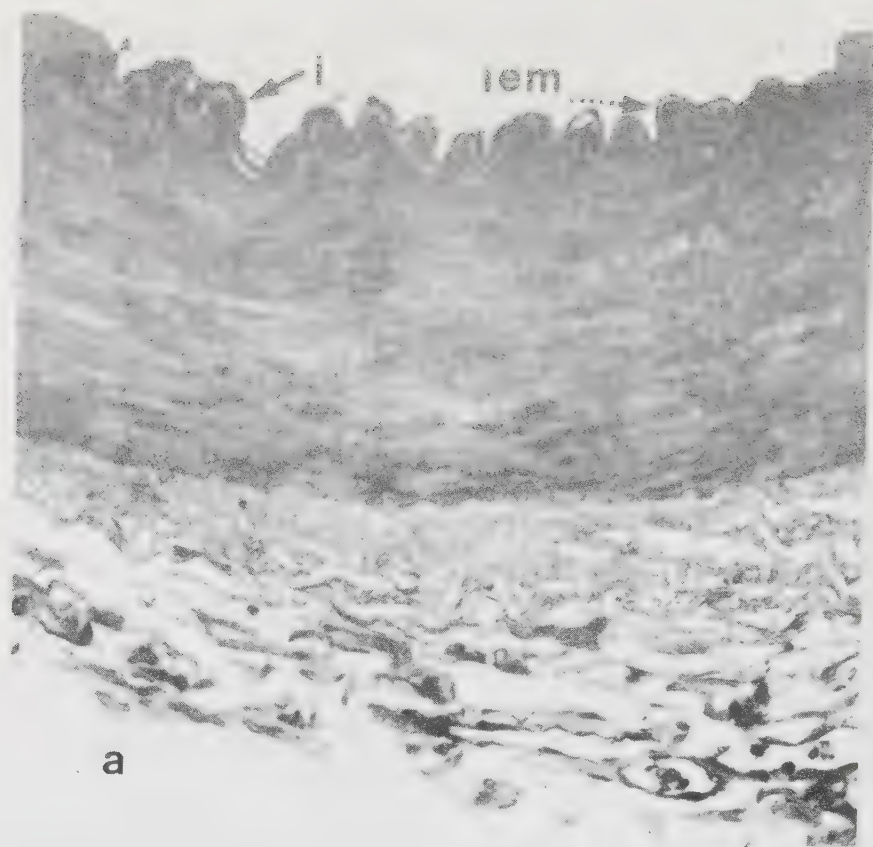
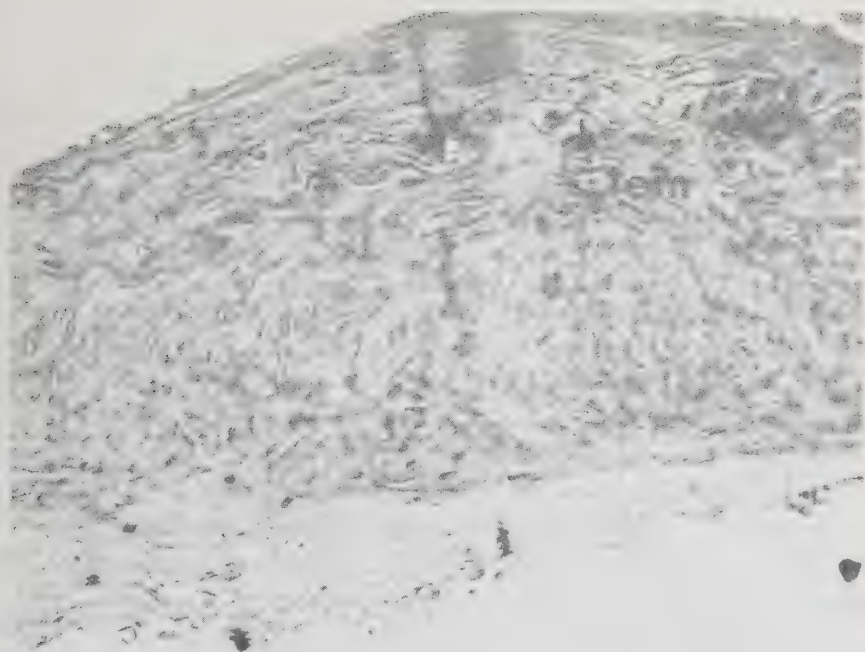


Fig 12. An antagonist experiment performed on a small vein (about 1 mm diam) obtained from within the soleus muscle in a 77 year old woman with severe leg ischemia due to atherosclerosis and diabetes mellitus. Three segments from the same vein specimen were investigated in parallel, using the first segment as a control (upper panel) and the second and third segment for testing the effect of increasing concentrations of the α_1 -receptor blocker prazosin (middle panel) and the α_2 -receptor blocker rauwolscine (lower panel). Noradrenaline stimulates both α_1 - and α_2 -receptors and its contractile responses are expressed as percentage of the K⁺ (124 mM)-induced contraction.





b

Fig 13. a. Branch of a small muscular artery from the groin region of a 53 year old healthy woman. The intimal layer ($i \rightarrow$) is thin. The internal elastic membrane ($iem \rightarrow$) is generally well preserved. b. Branch of a small muscular artery from the soleus muscle of a 59 year old man with severe leg ischemia (patient I, Table III). Compared to Fig 13a the intimal layer ($\leftarrow i \rightarrow$) is thicker and the internal elastic membrane ($iem \rightarrow$) is only focally preserved. Light micrographs. MacManus. x 225 .

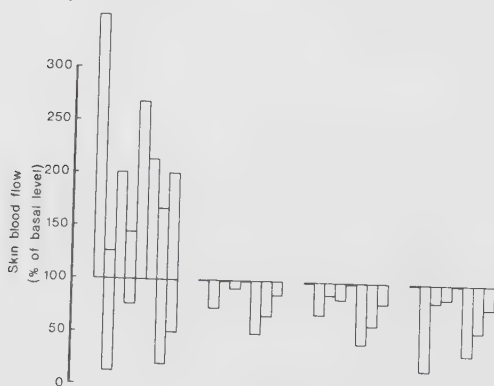
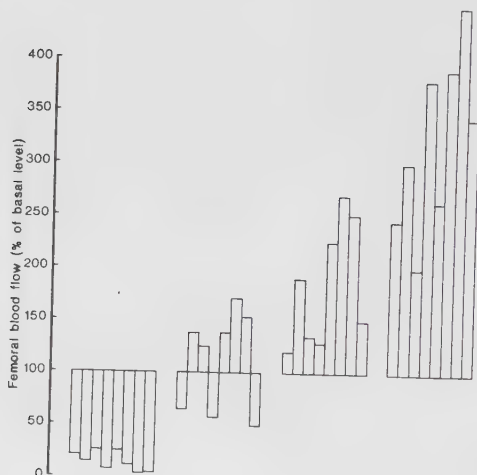
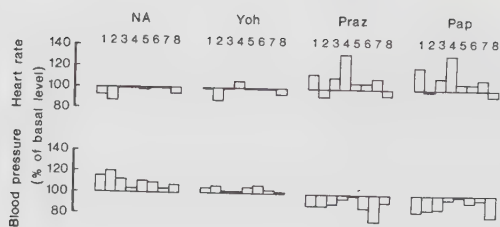


Fig 14. Registration of heart rate, systolic blood pressure and leg blood flow before and after intraarterially administered vasoactive drugs in 8 patients (1 - 8) undergoing femoro-popliteal by-pass procedure because of severe ischemia due to atherosclerosis. Epidural anesthesia was used in patients 1 - 4 and general anesthesia in patients 5 - 8. Femoral blood flow was measured with electromagnetic flowmeter by placing the flow probe on the deep femoral artery close to the bifurcation. Variations in skin blood flow (in signal voltage) were registered by laser doppler technique. The drugs were given in the common femoral artery during 10 s in the following order: noradrenaline (NA, $10 \text{ ml } 10^{-5} \text{ M}$), yohimbine (Yoh, $10 \text{ ml } 10^{-4} \text{ M}$), prazosin (Praz, $10 \text{ ml } 10^{-4} \text{ M}$) and papaverin (Pap, $40 \text{ mg} = 10 \text{ ml } 10^{-2} \text{ M}$). Peak responses are given (in % of basal levels).

Functional studies in vivo

In the peroperative experiments (Fig 14) NA caused a pronounced reduction of deep femoral artery blood flow (FBF) in all patients examined (i.e. also in patients 1 - 4 who had epidural anesthesia). The skin blood flow (SBF) as measured in signal voltage, increased abruptly the first seconds after the bolus dose of NA was given; when the maximum reduction in FBF was reached (after 30 - 90 s) SBF decreased rapidly back to the basal level (within 30 s) and below this level in three of the patients whereas the NA effects (blood pressure, FBF) disappeared gradually in 5 - 7 min.

Yohimbine increased FBF in five patients, and decreased it in three; no consistent changes in heart rate were observed, whereas a slight increase in blood pressure tended to occur. Prazosin and papaverin increased FBF in all patients. Both yohimbine, prazosin and papaverin had a similar effect on SBF, decreasing it or leaving it unchanged.

GENERAL DISCUSSION

Some of the results obtained in the present study deserve further comments, namely:

a) The reduction of the maximum response to NA in presence of prazosin and rauwolscine in omental veins (I)

b) The low slopes of the Schild plots generally seen except for prazosin in arteries (although significantly lower than 1 only for rauwolscine in the saphenous vein) (I, II, III)

c) The results obtained with oxymetazoline (I, II)

d) The results obtained with BHT-933 (III)

e) The calibres of the arteries used in vitro, although macroscopically small, are perhaps too large to be of any real physiological interest.

a). The reduction of the maximal response to NA in presence of prazosin and rauwolscine in omental veins suggest a noncompetitive antagonism. Results obtained by Stevens and Moulds (41, 61) for human hand vascular preparations and by De Mey and Vanhoutte (33) for dog saphenous vein suggest that α_2 -receptor stimulation produces a smaller maximum response compared with α_1 -receptor stimulation (62). Indeed, in rabbit uterus, although rich in α_2 -receptors, the α -receptor elicited contraction is mediated solely by the α_1 -receptors (45). Therefore, it seems possible that when a "critical" portion of the α_1 -receptors are blocked, the maximum response cannot be reached. One may also speculate that if both subtypes play a functionally important role in mediating contraction - and this seems to be the case in omental veins - stimulation of both subtypes may be necessary to reach

the maximum response; if then a substantial portion of one receptor subtype is blocked, stimulation of the other may not be sufficient to elicit the maximum response. This may be an explanation for the reduced maximum response seen in presence of the antagonists in the omental veins. In the groin and saphenous veins the α_1 -receptor population present seems to be of less significance than in omental veins (compare the NA concentration response-curves in presence of prazosin in the two regions), and this may explain why a significant depression of $E_{\max}$ was not seen in groin and saphenous veins (although there was a tendency to depression).

b). The slope of the Schild plot for rauwolscine in saphenous vein (III) was significantly lower than 1. Schild plots and pA_2 values are based on the assumption that the agonists and antagonists used interact with one type of receptor only (3, 58, 63).

Difficulties arise when an agonist can activate different receptor subtypes. Rauwolscine's α_2 - over α_1 selectivity is rather limited (30-fold or more, see 64) and over the concentration range required to construct a Schild plot, it is capable of antagonising both α_2 - and α_1 -receptors. The affinity of rauwolscine and yohimbine for α_1 -receptors, expressed in pA_2 values, has been shown to vary between 5.1 - 5.9 and 5.5 - 6.6 respectively (65). The highest concentrations of these

antagonists as used in the present study (10^{-5} M, 10^{-6} M) may have interacted with α_1 -receptors. Therefore, this lack of sufficient specificity may lead to a biphasic Schild plot which, when regarded as linear, will yield a slope of less than 1. In fact, rauwolscine seems to cause a biphasic Schild plot when antagonising NA's α_1 - and α_2 -receptor-mediated contractile effect in human omental arteries (65).

c). Oxymetazoline, although recognized as a selective α_2 -receptor agonist, seems to be more potent than the α_1 -receptor agonist phenylephrine, irrespective of the α -receptor subtype stimulated by the drugs (16). The postjunctional α -receptors in the rabbit aorta are of the α_1 -type (31); despite this, oxymetazoline was 25 times more potent than phenylephrine in this vessel (16). The rat basilar artery seems to lack contraction mediating α -receptors, and NA, phenylephrine and clonidine were unable to contract this artery (30). Oxymetazoline, however, was found to be relatively potent in producing contractions (30).

Furthermore, contractions induced by oxymetazoline in the present study (I, II) were slow in onset and extremely difficult to wash out. One may suggest that oxymetazoline is a non-reliable tool in α -receptor subtype research. It was therefore excluded in the last papers (III, V) of the present study.

d). BHT-933 is recognized as a rather selective α_2 -receptor agonist (66 - 70). The agonist had low potency in the saphenous

vein where it should be expected to be far more potent (III). The postjunctional α -receptors in middle cerebral artery of the cat was found to be of α_2 -type (37). In spite of this, BHT-933 was of very low potency (71). Thus, BHT-933 appears to be a drug of low potency regarding its interaction with human vascular postjunctional α_2 -receptors.

e). Although all vessels contribute to some degree to total peripheral resistance, it is a common impression that it is only the small arterioles which are concerned with the control of peripheral resistance (72). This impression has not, however, been confirmed by experimental data. Bohlen et al (73) investigating the pressure fall through the cremasteric muscle vasculature of the rat, found that the pressure in 100 μ m (inner diameter) arteries was already 50 mmHg below the mean aortic pressure, indicating that about 70% of the precapillary pressure fall occurs in vessels greater than 100 μ m in diameter. Fronck and Zweifach (74) found that in cat mesentery about 45% of the precapillary pressure drop occurs in vessels greater than 100 μ m in diameter. Folkow et al (75) studying the perfused rat hind-quarters under in vitro conditions, found that 25 - 30% of the precapillary resistance occurs in vessels proximal to those with a diameter of about 200 μ m, both when relaxed and when fully activated. It is therefore probable that a large portion of the vascular resistance is to be found proximal to the micro-circulation. Mulvany (72) suggests that the term "resistance vessel" should refer to all precapillary vessels less than 500 μ m

in inner diameter, although it must be remembered that this definition of "resistance vessel" covers a wide spectrum of vessel types where the larger ones probably play an important role in directing blood to those organs which the overall situation demands (e.g. defence reaction), while the smaller ones may be more concerned with the provision of local metabolic requirements. In the present study arteries with an inner diameter of at least 200 - 300 μm have been investigated, and they should be incorporated in the term larger resistance vessels.

Regional variations

The omental as well as the groin arteries were found to be endowed predominantly with α_1 -receptors occurring together with a small population of α_2 -receptors which seemed to be larger in omental than in groin arteries. On the other hand, the groin and saphenous veins seemed to be endowed predominantly with α_2 -receptors while the population of α -receptors in the omental veins seemed to be a more mixed one.

The physiological role of a regional variation of vascular α -receptors might be to direct the blood most appropriately in stress conditions with an increased activity in the sympathetic nerves and a high level of circulating catecholamines.

α -receptors in vitro and in vivo

Postjunctional α_2 -receptors in human arteries seem to be more easily demonstrated in vivo than in vitro (76, 77, V). An

explanation for this may be that the postjunctional α_2 -receptors on the arterial side are located predominantly in the arterioles which are far too small for direct in vitro studies.

Prazosin's inhibition of the NA-induced venocontraction was more pronounced in vivo than in vitro (IV). Indeed, prazosin 10^{-7} M in vitro did not significantly displace the NA concentration response-curve whereas prazosin in syringe concentration of 10^{-8} M (and thus further diluted by the blood before reaching the α -receptors in the vein wall) caused a significant inhibition of the NA-contraction in the veins of all six subjects; in two of the subjects no NA-contraction was elicited at all in presence of 10^{-8} M prazosin. Due to these in vivo results one may suggest the presence of a functionally important population of α_1 -receptors in the saphenous vein. Such a suggestion could not be made on basis of the in vitro results.

What is the reason for two subtypes of contraction mediating postjunctional α -receptors? Yamaguchi and Kopin (78) suggested that postsynaptic α -receptors located close to the neuroeffector junction were of the α_1 -subtype while those located at a distance away from the neuroeffector junction (i.e. extra-junctional α -receptors) were of the α_2 -subtype. The reason for this suggestion was their observation that pressor responses to exogenously administered catecholamines were selectively antagonized by α_2 -blockers and that the pressor responses elicited by sympathetic nerve stimulation were selectively antagonized by α_1 -

blockers. Langer et al (76, 79) using neuronal uptake inhibitors demonstrated that the responses to exogenous NA became susceptible to blockade by prazosin if the neuronal catecholamine uptake mechanism was inhibited with drugs such as cocaine. If the α_1 -receptors are concentrated at the neuroeffector junction and the α_2 -receptors are to be found mainly extrajunctionally, the α_1 -receptors will then be protected from exogenously administered NA by the neuronal uptake mechanism.

The innervation of blood vessels is found predominantly at the adventitial-medial border (80, 81, 82). Circulating catecholamines arriving via the blood stream thus have to traverse the media to reach the neuroeffector junction. If the hypothesis of extrasynaptic α_2 -receptors is true, it is possible that the α_2 -receptors are predominantly located in the inner layers of the media, while the α_1 -receptors are mainly located at the adventitial-medial border in close proximity to the noradrenergic nerves (76).

The vasoconstrictor responses to nerve stimulation should then be mediated predominantly by α_1 -receptors (and thus be blocked by prazosin) whereas responses to exogenous NA should be mediated predominantly through α_2 -receptors. If adrenaline is slightly more potent at α_2 - than α_1 -receptors as proposed by Langer and Shepperson (76), a hypothetical answer to the question why there are two contraction mediating postjunctional α -receptor subtypes in vascular smooth muscle is therefore that α_1 -receptors are present to mediate responses to neuronally released NA, and α_2 -

receptors to mediate the responses to circulating catecholamines released from the adrenal medulla. However, experiments have been done indicating that the final answer to this question has not yet been settled (27, 55, 83).

Difference in potency of NA between arteries and veins

A consistent finding in the present study (I, II, V) as well as in previous studies in man (41, 42, 84) is that NA is more potent in veins than in arteries. Possible explanations to this are that the amine uptake mechanisms may be different in arteries and veins, or that differences may exist regarding the content of α - and β -receptors.

Stevens and Moulds (85) have investigated the influence of the neuronal uptake mechanisms (uptake 1), extrajunctional uptake (uptake 2) and β -receptor mediated effects in human metacarpal veins and digital arteries. They found that the addition of propranolol ($4 \cdot 10^{-6}$ M) alone, or in combination with hydrocortisone ($4 \cdot 10^{-5}$ M), did not affect the responses to either NA or phenylephrine. The further addition of cocaine ($3 \cdot 10^{-5}$ M) slightly shifted the NA concentration response-curves to the left in both arteries and veins, but NA was still found to be more potent in veins than in arteries. They concluded therefore that the difference in sensitivity to NA between arteries and veins could not be explained by differences in NA uptake mechanisms or β -receptor content, but that there may be differences in the properties of the postsynaptic α -receptors in arteries and veins.

In the present study cocaine was used in the concentration of 10^{-6} M (to block uptake 1), since depression of $E_{\max}$ was observed in several experiments with the concentration of 10^{-5} M; no drugs (i.e. steroids) were used in order to block a possible extra-neuronal uptake of NA because such an uptake has been described to have little significance in several human isolated vessel preparations (22, 85). Propranolol in the concentration of 10^{-7} M was used to block the NA-interaction with possible β -receptors present. An increase of this propranolol concentration did not potentiate the NA response (I, II, III).

Arneklo-Nobin (84) investigating the adrenergic innervation in human hand vessels found that the sympathetic innervation was best developed in the arteries, where it formed a network of fibres located in the adventitia, superimposed on the smooth muscle media layer, but not penetrating into the media layer itself. The musculature in the wall of the veins was less well developed and demarcated and here the adrenergic nerve terminals coursed in among the superficial smooth muscle cells. The differences in sympathetic innervation between arteries and veins found in human hand vessels are characteristic also for the autonomic nerve supply in other vascular beds (80, 81). This does not contradict the suggestion by Langer (76) that α_2 -receptors have an extrajunctional location predominantly in the inner parts of media, it only suggests that sympathetic nerve terminals in veins are located nearer the intima than are the nerve terminals in arteries.

One may suggest that NA is more potent in veins than in arteries because veins have more postjunctional α_2 -receptors than arteries and those α_2 -receptors may be more sensitive to circulating NA than α_1 -receptors, because of their suggested extrajunctional location.

α -receptor subtypes and extra- and intracellular calcium

Contraction of vascular smooth muscle requires a certain threshold of calcium to be reached in the muscle cell to trigger the contractile process. It has recently been suggested that α_1 -receptors are less dependant upon extracellular calcium to produce a vasoconstrictor response than are the α_2 -receptors (70, 86). Indeed, the α_2 -receptor mediated vasoconstriction is antagonized by calcium entry blockers like nifedipine whereas α_1 -receptor mediated vasoconstriction is altered to a much lesser degree by such drugs (87). Nifedipine had a more marked antagonist effect on NA-induced contractions in isolated human crural veins than in arteries, but no such difference was seen in isolated human mesenteric veins and arteries (88). Prazosin was more potent than nifedipine in counteracting NA-induced contractions in isolated human mesenteric arteries and veins and in isolated human crural arteries; in isolated human crural veins, however, the effect of nifedipine was the more pronounced (88). This may indicate the significance of the postjunctional α_2 -receptors in human crural veins (II, III).

Nifedipine has been shown to have no effect on the ^3H -NA release (89). Calcium entry blockers open therefore up a new possibility of blocking postjunctional α_2 -receptor functions selectively without blocking prejunctional α_2 -receptors as is the case with the α_2 -receptor blockers. Thus, side effects such as tachycardia and diarrhoea (because of transmitter overflow) can be avoided (19).

Whether the prejunctional α_2 -receptors are identical with the postjunctional α_2 -receptors is not yet settled, although the present nomenclature implies that they do not differ in their sensitivity to the α -receptor agonists and antagonists most commonly in use (24). Hicks (90) has suggested that the α -receptor antagonist BE 2254 (HEAT) can discriminate between vascular pre- and postsynaptic α_2 -receptors in the pithed rat. If pre- and postsynaptic α_2 -receptors are different, it will be possible to synthesize still more α_2 -receptor subtype selective drugs, without side effects (tachycardia, diarrhoea) as mentioned above.

Does severe atherosclerosis alter vessel reactivity with regard to α -receptor drug treatment?

The main finding in study V was that the smaller arteries from ischemic legs had a great tendency to tachyphylaxis and showed great intra- and interindividual variation in their contractile response to potassium and α -receptor subtype selective agonists whereas the veins behaved like groin and saphenous veins in

healthy subjects (II, III). However, the substantial effects of NA, prazosin and papaverin in vivo in all patients show that far from all vessels in ischemic legs are rigid non-reactive structures beyond pharmacological manipulation. The effects on femoral blood flow of prazosin and especially papaverin show that there is far more vasodilatation to obtain than that caused by epidural anesthesia. The patients with epidural anesthesia had dilated subcutaneous veins. After NA-injection these veins contracted heavily and "disappeared". In one patient with extremely dilated subcutaneous veins because of the epidural anesthesia the venoconstriction after NA was so pronounced that a furrow in the skin marking out the whole length of the long saphenous vein could be seen; when yohimbine was injected the same vein dilated to initial size in spite of the decrease in femoral blood flow in that patient. Epidural anesthesia is probably without value as a protection against excessive vasoconstriction elicited by circulating catecholamines e.g. in stress conditions.

α -receptor subtypes and venous physiology

One may suggest that the most interesting possibility with selective influence on α -receptor subtype functions, concerns venous physiology. About seventy per cent of the blood volume is located within the veins. The precapillary resistance vessels are in a constant state of partial constriction which is exerted through inherent myogenic activity which is also manifest when

extrinsic nervous influences or humoral excitatory factors are absent. The venous capacitance vessels, on the other hand, exhibit little myogenic activity; their tone is dependent instead upon extrinsic sympathetic vasoconstrictor influences (81). The blood pressure in the small venules and veins is the driving pressure for venous return and it is continuously modified by the sympathetic tone. When this venous driving pressure is the same as the right atrial pressure, the venous return is zero and there will be no cardiac output.

An interesting suggestion is that a carefully titrated systemic infusion of NA selectively may increase the venous tone, since NA appears to be 5 - 10 times more potent in veins than in arteries. In this way it should be possible to increase venous return and cardiac output without increasing the cardiac afterload. Conversely one may suggest that a selective and controlled reduction of the venous tone (e.g. by calcium entry blockers and/or α_2 -receptor blockers) may be an important way of reducing cardiac preload. A combination of an α_1 -blocker (e.g. prazosin) and a calcium entry blocker (e.g. nifedipine) may be a very potent hypotensive drug as it may reduce both α_1 - and α_2 -receptor mediated vascular tone without directly increasing the sympathetic outflow.

CLINICAL APPLICATION AND FUTURE ASPECTS

The potential significance of α_1 - and α_2 -receptor mediated effects in the regulation of blood pressure and cardiac output

has already been discussed and should be of particular interest in the treatment of hypertension and cardiac failure (especially acute cardiac failure).

Table IV suggests some situations of surgical interest which may profit from greater knowledge about vascular α -receptor subtypes .

Table IV

Some situations of surgical interest which may profit from greater knowledge about vascular α -receptor subtypes.

Surgery for pheochromocytoma

Shock

Venous thrombosis prophylaxis

Vasospastic disorders

Pharmacangiography

Severe leg ischemia

Vein by-pass surgery

Microvascular surgery

Bowel surgery

Surgery for pheocromocytoma

Anesthesia and any form of operative procedure can readily precipitate a massive release of adrenaline, noradrenaline or both. The patient may in such cases present convulsive seizures, cardiac arrhythmias and systolic blood pressures in excess of 300 mmHg. He may sustain a paralytic stroke, or sudden death (91). Preoperative preparation of these patients with adrenoceptor blocking drugs represents a therapeutic breakthrough. The commonly used α -receptor blockers are phenoxybenzamine and phentolamine. Phenoxybenzamine is a slow acting and irreversible α -receptor blocker (i.e. a relatively stable bond is formed with the α -receptors). This makes phenoxybenzamine difficult to handle; with a sudden massive release of catecholamines, dangerous symptoms can still be attained because of the slow action. Furthermore, after the extirpation of the pheocromocytoma there is often difficulty to keep an adequate blood pressure, and now phenoxybenzamine is of negative value. Phentolamine is a competitive α -receptor antagonist. An unexpected massive release of catecholamines may easily compete out this antagonist which therefore cannot give a sure protection alone.

One may suggest that an effective way of gaining satisfactory control in such cases would be a cocktail of prazosin and a calcium entry blocker like nifedipine. Calcium entry blockers should in this respect be valuable because of their indirect blockade of α_2 -receptor functions. If severe tachycardia super-

venes, a β -receptor blocker (e.g. propranolol) should be added, but not until an adequate blockade of α -receptor functions has been established.

Shock

Since there seems to be a predominance of α_2 -receptors in veins and of α_1 -receptors in arteries, one may suggest that e.g. the development of a drug which stimulates α_2 -receptors and blocks α_1 -receptors and does not cross the blood-brain barrier, should be a valuable tool as a complement to the main therapeutic actions in many shock conditions where the primary aim is to increase the venous return (i.e. contractions of veins) and the tissue perfusion (i.e. dilatation of precapillary resistance vessels).

Venous thrombosis prophylaxis

Several studies (92 - 97) have shown that dihydroergotamine (DHE) in combination with heparin is more effective in prevention of postoperative thrombotic complications than heparin alone. DHE is a relatively unselective partial α -receptor agonist. Lindblad et al (98) found a 20% reduction of venous area and a 40% venous blood flow rate increase in the groin with DHE, leaving the arterial area and blood flow rate unchanged. They also achieved a decreased reserve venous volume by DHE whereas no major effect was found on isotope or contrast clearance from the calf. One may suggest that a more selective α_2 -receptor agonist

than DHE may be more effective in increasing the flow rate in the veins of the lower extremity. DHE has also been shown to increase the concentration of antithrombin III and counteract the decrease in fibrinolytic activity in vein walls which normally occurs postoperatively (99). Together with the increased venous flow rate this might explain the potentiating effect of DHE in prophylactic treatment with low dose heparin.

Vasospastic disorders

Stevens and Moulds (41) have shown that postjunctional α -receptors of both subtypes are present in human hand arteries and veins. Functional blockade of α -receptors has a place in the treatment of severe ischemia with gangrene in Raynaud's disease, in combination with blockade of serotoninreceptors which also may mediate vasoconstriction in human hand vessels (84).

Pharmacangiography

Optimal angiographic demonstrations of organic stenosis or occlusions can only be obtained if simultaneously present vasospasm is eliminated. Arneklo-Nobin (84) concluded that body warming in combination with an injection of 4 mg of the α -receptor blocker phentolamine, by eliminating sympathetically induced vasospasm, gave a normal blood flow and sufficient vasodilation in upper extremity angiography for visualization of the pertinent areas for a decision about reconstructive vascular surgery.

The problem of obtaining a reliable routine angiogram in order to make a satisfactory decision for or against reconstructive surgery in the ischemic leg, is a more difficult one. All too often the patient with "no run off" as judged from the angiogram, does have patent vessels for a by pass procedure when explored surgically, i.e. the X-ray exposition must have failed. It should be possible to "arrest" the X-ray contrast in the leg for some time by a selective venoconstriction (e.g. by an α_2 -receptor agonist or perhaps better by a selective NA-elicited venoconstriction).

Severe leg ischemia

In severely atherosclerotic legs or in iliofemoral venous thrombosis with phlegmasia cerulea dolens or after by-pass operations for limb salvage, the patients may have high levels of circulating catecholamines due to for example severe pain or anxiety. This may reduce the femoral blood flow because of arteriolar vasoconstriction, but perhaps more important, give a pronounced venoconstriction which might be critical for the run off. Pharmacotherapy (including α_1 - and α_2 -receptor blockade, anxiolytic and analgesic therapy) might therefore be of special importance in such cases. However, α -receptor blockade as used in this study (V), decreased the skin blood flow, and this is important to consider in the chronic treatment of patients with skin ulcers or gangrene.

Vein by-pass surgery

The long saphenous vein is the graft of choice in coronary and femoropopliteal by-passes as well as in peripheral vascular trauma surgery. This vein was found to be endowed with predominantly α_2 -receptors postjunctionally (III) although α_1 -receptors also seem to be of importance (IV). Circulating catecholamines are suggested to act principally on α_2 -receptors (76). The observations made in study V surely make it clear that catecholamines are able to elicit pronounced contraction in this vein in vitro as well as in vivo (in spite of epidural anesthesia). α -receptor blockade may have a place to counteract graft spasm in connection with surgery. Because the vein graft is denervated it may be supersensitive to circulating catecholamines. Holmberg et al (43) studying the contractile response of isolated human vessels after storage in chilled Krebs solution, found that both the sensitivity and the contractile force to noradrenaline increased considerably during the first days of storage. In denervated vessels the intramural autonomic nerve plexa will degenerate, and so will the neuronal uptake of circulating noradrenaline. This may explain the supersensitivity to noradrenaline. The number of functioning receptors on the denervated vascular smooth muscle cells may also be upregulated (43). Because the presynaptic α_2 -receptors will disappear in denervated vessels, there will be no transmitter overflow following α_2 -blockers which might be particularly valuable to counteract "stress spasm" in vein grafts.

Microvascular surgery

Robins (49) has suggested that α -receptor blockade is of value in connection with free skin flap transfer surgery. One may suggest a similar explanation for the value of such blockade in free skin flaps (where all vessels are denervated) as in vein grafts (see the discussion under vein by-pass surgery).

Bowel surgery

Ischemia is a predisposing factor in colonic anastomotic dehiscence, and may result from microcirculatory failure. Foster et al (100) found that oral α -receptor blockade was beneficial in colonic healing of rat colon anastomoses rendered ischemic by tying adjacent mesenteric vessels.

Pharmacological receptor classification in the future?

In the future molecular biology will probably contribute to a more exact receptor classification than can be obtained by pharmacological methods, in which imaginary receptors are defined on basis of drug effects. However, receptor classification is of real interest in clinical medicine only if it contributes to clinical understanding and therapy. The above mentioned examples show that such a contribution is available and will continue to aid the two main pathways of therapy, drugs and surgery. Therefore, the development of pharmacological classification of receptors will continue to be of the utmost importance.

GENERAL CONCLUSIONS

*Human peripheral blood vessels have a mixed population of contraction mediating postjunctional α -receptors with a predominance of α_1 -receptors in the arteries and a predominance of α_2 -receptors in the veins, although regional variations occur.

*Noradrenaline is more potent in veins than in arteries.

*In vitro small arteries, but not veins, from ischemic atherosclerotic legs show a great tendency of tachyphylaxis and a great intra- and interindividual variation in contractile capacity as compared with small arteries and veins in healthy legs.

*In vivo noradrenaline/papaverin elicited pronounced vasoconstriction/vasodilatation in all atherosclerotic legs tested, indicating that the smallest arteries and arterioles are well reactive structures also in severe atherosclerotic legs.

*The α_1 -receptor blocker prazosin does not significantly antagonize the NA-induced contraction of the saphenous vein in vitro, but is a potent antagonist in this respect in vivo. The α_2 -receptor blocker yohimbine is a potent antagonist against the NA-induced contraction of the saphenous vein both in vitro and in vivo.

*Prazosin increases the femoral artery blood flow in ischemic legs, whereas yohimbine does not show any consistent effects in this respect, decreasing it slightly in some patients and increasing it slightly in other patients. α -receptor blocking drugs decrease or do not affect the skin blood flow in ischemic legs.

*Epidural anesthesia is unable to abolish vasoconstriction elicited by circulating noradrenaline.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to:

Professor Stig Bengmark, my chief, for his never failing enthusiasm in research.

Associate professor Lars Norgren for initiating this study, and for guiding me throughout the work.

Professor Karl-Erik Andersson for invaluable support and constructive criticism.

My co-authors Trygve Sjöberg, Tor Skärby, Jan Castenfors and Per Alm for friendly collaborations and fruitful discussions.

Inger Carlsson for the skilful and sometimes endless typing and retyping of tables and manuscripts.

Ing-Britt Mårtensson, Eva Edestad, Gunilla Sernelin, Else-Britt Granbom and Lotta Paulsson for expertize illustrative, secreterial and technical assistance.

Per Johnson, friend and printer.

All patients and volunteers who participated in the study.

My friends in the Departments of Surgery and Clinical Pharmacology.

This thesis was supported financially by Sven and Dagmar Sahlen's Foundation.

REFERENCES

1. Langley MA (1878) On the physiology of the salivary secretion. *J Physiol* 1:339-369
2. Goth A (1981) Medical Pharmacology. Principles and Concepts (10th edition), pp.7-13. The C.V. Mosby Company.
3. Ruffolo, Jr RR (1982) Review. Important concepts of receptor theory. *J Auton Pharmac* 2:277-295
4. Ariens EJ (1983) Receptors: the materialization of a concept. *Pharmaceutisch Weekblad Scientific Edition* 5:121-128
5. Ganong WF (1983) Review of Medical Physiology (11th edition), pp. 25-29. Lange Medical Publications.
6. Kistler J, Stroud RM, Klymkowski MW, Lalancette RA, Fairclough RH (1982) Structure and function of an acetylcholine receptor. *Biophys J* 37:371-383
7. Mc Namee MG, Ochoa ELM (1982) Reconstitution of acetylcholine receptor function in model membranes. *Neuroscience* 7:2305-2319
8. Langley JN (1905) On the reaction of cells and nerve endings to certain poisons, chiefly as regards the reaction of striated muscle to nicotine and to curare. *J Physiol* 33:374
9. Ahlquist RP (1948) A study of the adrenotropic receptors. *Amer J Physiol* 153:586-600
10. Carrier GO, Schlafer M (1984) Raymond P. Ahlquist 26 July 1914 - 15 April 1983. *Trends Pharmacol Sci* 5:41-44

11. Lands AM, Arnold A, McAuliff JP, Luduena FP, Brown TG.Jr (1967) Differentiation of receptor systems activated by sympathomimetic amines. *Nature* 214:597-598
12. von Euler US (1951) The nature of adrenergic nerve mediators. *Pharmac Rev* 3:247-277
13. Langer SZ (1974) Presynaptic regulation of catecholamine release. *Biochem Pharmacol* 23:1793-1800
14. Berthelsen S, Pettinger WA (1977) A functional basis for classification of α -adrenergic receptors. *Life Sci* 21:595-606
15. Wikberg JES (1978) Pharmacological classification of adrenergic α -receptors in the guinea pig. *Nature* 273:164-166
16. Wikberg JES (1979) The pharmacological classification of adrenergic α_1 and α_2 receptors and their mechanisms of action. Thesis. *Acta Physiol Scand*, Suppl. 468
17. Starke K, Langer SZ (1979) A note on terminology for presynaptic receptors. In: *Presynaptic receptors* (Ed. SZ Langer, K Starke & M L Dubocovich), pp.1-3. Pergamon press, Oxford.
18. Starke K (1981) α -Adrenoceptor subclassification. *Rev Physiol Biochem Pharmacol* 88:199-236
19. Langer SZ, Shepperson NB (1981) Subclassification of α -adrenoceptors into α_1 - and α_2 -categories: relevance to antihypertensive therapy. *New Trends in Arterial Hypertension. Intern Symposium no 17*. Ed. M Worcel et al. Elsevier/North Holland Biomedical Press, pp. 73-85

20. Langer SZ (1981) Presynaptic regulation of the release of catecholamines. *Pharmacol Rev* 32:337-362
21. Langer SZ, Shepperson NB (1982) Prejunctional modulation of noradrenaline release by α_2 -adrenoceptors: physiological and pharmacological implications in the cardiovascular system. *J Cardiovasc Pharmacol* 4:S35-S40
22. Janssens W, Verhaeghe R (1983) Modulation of the concentration of noradrenaline at the neuro-effector junction in human saphenous vein. *Br J Pharmacol* 79:577-585
23. Charney DS, Heninger GR, Sternberg DE (1982) Assessment of α_2 -adrenergic autoreceptor function in humans: effects of oral yohimbine. *Life Sci* 30:2033-2041
24. Skärby T (1984) Pharmacological properties of prejunctional α -adrenoceptors in isolated feline middle cerebral arteries; comparison with the post-junctional α -adrenoceptors. *Acta Physiol Scand*, in press.
25. van Meel JCA, Timmermans PBMW, van Zwieten PA (1983) α_1 - and α_2 -Adrenoceptor stimulation in the isolated perfused hindquarters of the rat: an in vitro model. *J Cardiovasc Pharmacol* 5:580-585
26. Madjar H, Docherty JR, Starke K (1980) An examination of pre- and postsynaptic α -adrenoceptors in the autoperfused rabbit hindlimb. *J Cardiovasc Pharmacol* 2:619-627
27. Gardiner JC, Peters CJ (1982) Postsynaptic α_1 - and α_2 -adrenoceptor involvement in the vascular responses to neuronally released and exogenous noradrenaline in the hindlimb of the dog and cat. *Eur J Pharmacol* 84:189-198

28. Langer SZ, Massingham R, Shepperson NB (1980) Presence of postsynaptic α_2 -adrenoceptors of predominantly extrasynaptic location in the vascular smooth muscle of the dog hind limb. Clin Sci 59:225S-228S
29. Llenas J, Massingham R (1983) A comparison of the effects of verapamil and cinnarizine upon responses elicited by selective α_1 - and α_2 -adrenoceptor agonists in the . autoperfused canine hindlimb. Eur J Pharmacol 87:53-59
30. Högestätt ED (1984) On the role of calcium in depolarization-induced and receptor- mediated contractions in isolated cerebral and peripheral arteries. Doctoral Dissertation, University of Lund, Sweden, pp. V:1-16
31. Ruffolo RR, Waddell JE, Yaden EL (1982) Heterogeneity of postsynaptic alpha adrenergic receptors in mammalian aortas. J Pharmacol Exp Ther 221:309-314
32. Starke K, Endo T, Taube HD (1975) Relative pre- and postsynaptic potencies of α -adrenoceptor agonists in the rabbit pulmonary artery. Naunyn Schmiedeberg's Arch Pharmacol 291:55-78
33. De Mey J, Vanhoutte PM (1981) Uneven distribution of postjunctional α_1 - and α_2 -like adrenoceptors in canine arterial and venous smooth muscle. Circulat Res 48:875-884
34. Hicks PE (1983) α -Adrenoceptor-mediated phasic and tonic activity in rat portal vein in vitro. J Auton Pharmacol 3:97-106

35. Docherty JR, Starke K (1981) Postsynaptic α -adrenoceptor subtypes in rabbit blood vessels and rat anococcygeus muscle studied in vitro. *J Cardiovasc Pharmacol* 3:854-866
36. Ruffolo RR, Jr, Waddell JE, Yaden EL (1981) Postsynaptic alpha adrenergic receptor subtypes differentiated by yohimbine in tissues from the rat. Existence of alpha-2 adrenergic receptors in rat aorta. *J Pharmacol Exp Ther* 217:235-240
37. Skärby TVC, Andersson K-E, Edvinsson L (1983) Pharmacological characterization of postjunctional α -adrenoceptors in isolated feline cerebral and peripheral arteries. *Acta Physiol Scand* 117:63-73
38. Sakakibara Y, Fujiwara M, Muramatsu I (1982) Pharmacological characterization of the alpha adrenoceptors of the dog basilar artery. *Naunyn-Schmiedeberg's Arch Pharmacol* 319:1-7
39. Constantine JW, Lebel W, Archer R (1982) Functional postsynaptic α_2 - but not α_1 -adrenoceptors in dog saphenous vein exposed to phenoxybenzamine. *Eur J Pharmacol* 85:325-329
40. Shepperson NB, Langer SZ (1981) The effects of the 2-amino-tetrahydronaphthalene derivative M7, a selective α_2 -adrenoceptor agonist in vitro. *Naunyn-Schmiedeberg's Arch Pharmacol* 318:10-13
41. Stevens MJ, Moulds RFW (1981) Heterogeneity of post-junctional α -adrenoceptors in human vascular smooth muscle. *Arch Int Pharmacodyn* 254:43-57
42. Glusa E, Markwardt F (1983) Characterization of postjunctional α -adrenoceptors in isolated human femoral veins and arteries. *Naunyn-Schmiedeberg's* 323:101-105

43. Holmberg JT, Thulesius O, Gjores JE (1983) Contractile response of isolated human vessels with special regard to stretch and storage. *Gen Pharmacol* 14:77-79
44. Williams LT, Lefkowitz RJ (1978) Receptor binding studies in adrenergic pharmacology. Raven Press, New York
45. Hoffman EB, Lavin TN, Lefkowitz RJ, Ruffolo RR, Jr (1981) Alpha adrenergic receptor subtypes in rabbit uterus: mediation of myometrial contraction and regulation by estrogens. *J Pharmacol Exp Ther* 219, No2:290-295
46. Schumann H-J, Lues I (1983) Postjunctional α -adrenoceptors in the isolated saphenous vein of the rabbit Characterization and influence of angiotensin. *Naunyn-Schmiedeberg, s Arch Pharmacol* 323:328-334
47. Taylor I, Silber SJ (1979) Free vascularized transfers of skin flaps, bone, nerve and muscle. In: *Microsurgery*, Williams & Wilkens, Baltimore/London
48. Ohmori K, Harii K (1979) Restoration of skin cover by free flap transfer. In: *Operative Surgery, Fundamental International Techniques, Plastic Surgery*. Butterworths & Co, London.
49. Robins DW (1983) Alpha-adrenergic blockade in prolonged surgery. *Research and Clinical Forums*, 5, no 2, 1983.
50. Högestätt ED, Andersson K-E, Edvinsson L (1983) Mechanical properties of rat cerebral arteries as studied by a sensitive device for recording of mechanical activity in isolated small blood vessels. *Acta Physiol Scand* 117:49-61

51. Castenfors J, Felding A (1984) A simple technique for measuring changes in size of subcutaneous veins. In manuscript.
52. Thulesius O (1983) Chronic venous insufficiency and varicose veins - pathophysiological considerations. *Läkartidningen* 80:1794-1796, 1800-1801
53. Thulesius O, Gjöres JE (1974) Reactions of venous smooth muscle in normal men and patients with varicose veins. *Angiology* 25:145-154
54. Tenland T (1982) On laser doppler flowmetry. Methods and microvascular applications. Thesis. University of Linköping.
55. McGrath JC (1982) Evidence for more than one type of postjunctional α -adrenoceptor. *Biochem Pharmacol* 31:467-484
56. Nilsson G, Tenland T, Öberg PÅ (1980) A new instrument for continuous measurement of tissue blood flow by light beating spectroscopy. *IEEE Transactions on Biomed Engineering* BME-27, 1
57. Nilsson G, Tenland T, Öberg PÅ (1980) Evaluation of a laser doppler flowmeter for measurement of tissue blood flow. *IEEE Transactions on Biomed Engineering* BME-27, 10
58. Arunlakshana O, Schild HO (1959) Some quantitative uses of drug antagonists. *Brit J Pharmacol* 14:48-58
59. Schild HO (1949) pA_x and competitive drug antagonism. *Brit J Pharmacol* 4:277-280
60. Kenakin TP (1981) The Schild regression in the process of receptor classification. *Can J Physiol Pharmacol* 60:249-265

61. Stevens MJ, Moulds RFW (1982) Are the pre- and postsynaptic α -adrenoceptors in human vascular smooth muscle atypical? *J Cardiovasc Pharmacol* 4:S129-S133
62. Flavahan NA, McGrath JC (1984) Are human vascular α -adrenoceptors atypical? *J Cardiovasc Pharmacol* 6:208-210
63. Furchgott RF (1972) The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. In: *Handbook of Experimental Pharmacology* (Ed. H Blaschko & E Muscoll), pp.283-355. Springer Verlag, New York.
64. Ruffolo RR, Jr (1983) Alpha-adrenoceptors. In: *Neuroreceptors in Health & Disease* (Ed. J Marhawa and W Andersson) S. Karger AG, Basel.
65. Skärby T (1984) α -Adrenoceptors and calcium dependent contractions in isolated cerebral and peripheral arteries. Doctoral Dissertation, University of Lund, Sweden.
66. Kobinger W, Pichler L (1980) Relation between central sympathoinhibitory and peripheral pre- and postsynaptic α -adrenoceptors as evaluated by different clonidine-like substances in rats. *Naunyn-Schmiedeberg's Arch Pharmacol* 315:21-27
67. Kobinger W, Pichler L (1981) α_1 -adrenoceptor blockade by α_2 -adrenoceptor agonists in the isolated perfused hindquarter of rats. *Eur J Pharmacol* 72:113-115

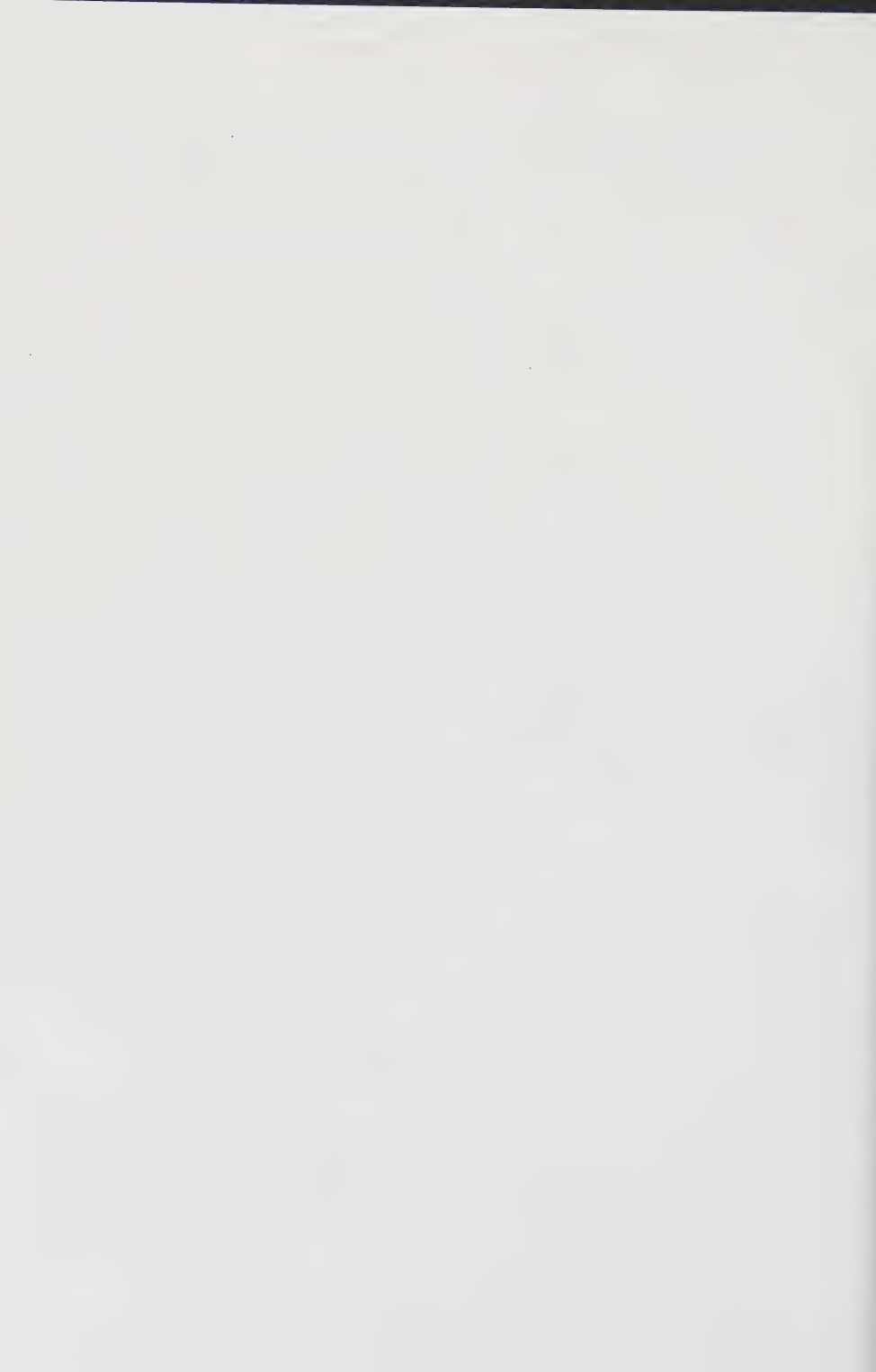
68. Timmermans PBMWM, van Meel JCA, van Zwieten PA (1980) Evaluation of the selectivity of α -adrenoceptor blocking drugs for postsynaptic α_1 - and α_2 -adrenoceptors in a simple animal model. *J Auton Pharmacol* 1:53-60
69. van Meel JCA (1982) The vascular postjunctional α_2 -adrenoceptor. Thesis. Rodopi, Amsterdam.
70. Timmermans PBMWM, van Zwieten PA (1982) α_2 Adrenoceptors: Classification, localization, mechanisms, and targets for drugs. *J of Med Chem* 25:1389-1401
71. Skärby T, Personal communication.
72. Mulvany MJ (1983) Do resistance vessel abnormalities contribute to the elevated blood pressure of spontaneously hypertensive rats? *Blood Vessels* 20:1-22
73. Bohlen H-G, Gore RW, Hutchins PM (1977) Comparison of microvascular pressures in normal and spontaneously hypertensive rats. *Microvasc Res* 13:125-130
74. Fronek K, Zweifach BW (1975) Microvascular pressure distribution in skeletal muscle and the effect of vasodilatation. *Am J Physiol* 228:791-796
75. Folkow B, Hallbäck M, Jones JV, Sutter K (1977) Dependence of external calcium for noradrenaline contractility of resistance vessels in spontaneously hypertensive and renal hypertensive rats, as compared with normotensive controls. *Acta Physiol Scand* 101:84-97
76. Langer SZ, Shepperson NB (1982) Recent developments in vascular smooth muscle pharmacology: the post-synaptic α_2 -adrenoceptor. *Trends Pharmacol Sci* 3:440-444

77. Elliott HL, Reid JL (1983) Evidence for postjunctional vascular α_2 -adrenoceptors in peripheral vascular regulation in man. *Clin Sci* 65:237-241
78. Yamaguchi I, Kopin IJ (1980) Differential inhibition of α_1 - and α_2 -adrenoceptor mediated pressor responses in pithed rats. *J Pharmacol Exp Ther* 214:275-281
79. Langer SZ, Shepperson NB (1982) Postjunctional α_1 - and α_2 -adrenoceptors: preferential innervation of α_1 -adrenoceptors and the role of neuronal uptake. *J Cardiovasc Pharmacol* 4:S8-S13
80. Ehinger B, Falck B, Sporrang B (1966) Adrenergic fibres to the heart and to peripheral vessels. *Symp. electr. Activ. Innerv. Blood Vessels*, Cambridge 1966; *Bibl. anat.* (Karger, Basel/New York) 8:35-45
81. Owman C (1980) Vascular autonomic nerves and corresponding receptors, with particular reference to neurogenic mechanisms of vasodilatation. *Progress Pharmacol* 3-4:47-68
82. Gray's anatomy (35th edition, 1973), Longman, New York.
83. Docherty JR, Starke K (1981) Postsynaptic α -adrenoceptor subtypes mediating nerve-evoked contractions in rabbit blood vessels in vitro. Abstract. Proceedings of the Brit. Pharmacol. Soc., p.803
84. Arneklo-Nobin B (1983) The white cold hand. Physiologic, angiographic and pharmacologic study on the hand circulation with special reference to Raynaud's phenomenon. Thesis. Bulletin no 37 from the Department of Surgery, University of Lund, Sweden.

85. Stevens MJ, Moulds RFW (1980) Are neuronal uptake mechanisms responsible for the difference in sensitivity to noradrenaline between human arteries and veins? *Clin Exp Pharmacol Physiol* 7:139-145
86. van Meel JCA, De Jonge A, Kalkman HO, Wilffert B, Timmermans PBMWM, van Zwieten PA (1981) Vascular smooth muscle contraction initiated by postsynaptic α_2 -adrenoceptor activation is induced by an influx of extracellular calcium. *Eur J Pharmacol* 69:205-208
87. van Meel JCA, De Jonge A, Kalkman HO, Wilffert B, Timmermans PBMWM, van Zwieten PA (1981) Organic and inorganic calcium antagonists reduce vasoconstriction in vivo mediated by postsynaptic α_2 -adrenoceptors. *Naunyn-Schmiedeberg's Arch Pharmacol* 316:288-293
88. Lederballe Pedersen O, Mikkelsen E, Andersson K-E (1979) Comparison of the in vitro effects of prazosin, nifedipine and dihydralazine in isolated human mesenteric and crural vessels. *Arch int Pharmacodyn Ther* 241:224-234
89. Högestätt ED, Andersson K-E, Edvinsson L (1982) Effects of nifedipine on potassium-induced contraction and noradrenaline release in cerebral and extracranial arteries from rabbit. *Acta Physiol Scand* 114:283-296
90. Hicks PE (1981) Antagonism of pre and postsynaptic α -adrenoceptors by BE 2254 (HEAT) and prazosin. *J Auton Pharmacol* 1:397-391
91. Scott HW Jr (1978) The panic syndrome: Pheocromocytoma. In *Surgical Endocrinology. Clinical syndromes* (Eds. S.R. Friesen and R.E. Bolinger), pp. 102-119, J.B. Lippincott Company, Philadelphia.

92. Sasahara AA, DiSerio FJ (1983) Prophylaxis of postoperative deep vein thrombosis with a dihydroergotamine-heparin combination: A multicenter trial. Abstract. *Thromb Haemost* 50:9
93. Breddin K, Häring R, Koppenhagen K (1983) Prevention of postoperative thrombotic complications with small doses of heparin and dihydroergotamine. Abstract. *Thromb Haemost* 50:304
94. Rajah SM, Salter MCP, Donaldson DR, Crow MJ, Rao RS, Watson DA (1983) A clinical trial comprising 2500 IU heparin in the prophylaxis of deep venous thrombosis following thoracic surgery. Abstract. *Thromb Haemost* 50:9
95. Ammann JF, Aebi B (1983) Risk of postoperative hemorrhage in prophylaxis of thromboembolism: Comparison of low-dose heparin to heparin DHE 2500. Abstract. *Thromb Haemost* 50:65
96. Veth G (1983) Prevention of postoperative thrombo-embolism by a combination of heparin with dihydro-ergotamine or with sulphinpyrazone. *Thromb Haemost* 50:471
97. Gruber UF, Schenker AB, Kradolfer ST, Gerber H (1983) Cumarin, dextran or heparin-dihydroergotamine for prevention of fatal pulmonary embolism after fracture of the hip? Abstract. *Thromb Haemost* 50:248
98. Lindblad B (1983) Combination prophylaxis of postoperative deep vein thrombosis. An evaluation of three combined regimens. Doctoral Dissertation. Malmö, Sweden
99. Svanberg L, Bernstein K, Kullander S, Åstedt B (1980) Effect of dihydroergotamine on coagulation factors and components of the fibrinolytic system. *Acta Obstet Gynecol Scand* 59:251-253

100. Foster ME, Brennan SS, Leaper DJ (1984) Ischaemia in colonic anastomoses - beneficial effect of a vasodilator.
Abstract.Eur Surg Res 16 (S1):17



Pharmacological characterization of postjunctional α -adrenoceptors in isolated human omental arteries and veins

STIG STEEN, TOR V. C. SKÄRBY, LARS NORGREN
and KARL-ERIK ANDERSSON

Departments of Clinical Pharmacology and Surgery, University of Lund, Sweden

STEEN, S., SKÄRBY, T. V. C., NORGREN, L. & ANDERSSON, K.-E.: Pharmacological characterization of postjunctional α -adrenoceptors in isolated human omental arteries and veins. *Acta Physiol Scand* 1984, 120: 109–116. Received 6 May 1983. ISSN 0001-6772. Departments of Clinical Pharmacology and Surgery, University of Lund, Sweden.

The α -adrenoceptors in human omental arteries and veins were characterized and compared. In the arteries both prazosin (pA_2 9.48) and rauwolscine (pA_2 7.19) displaced the noradrenaline (NA) concentration-response (cr) curve towards higher concentrations without reduction of maximum. Neither clonidine, nor oxymetazoline had any consistent contractile effects. Phenylephrine had a lower potency than NA, but a similar intrinsic activity. In the veins, both prazosin (pA_2 9.72) and rauwolscine (pA_2 8.11) displaced the NA cr-curve towards higher concentrations, but also significantly depressed maximum. Clonidine and oxymetazoline contracted veins from 3 out of 7 and 4 out of 6 patients, respectively. Their pD_2 -values were similar to that of NA, but their intrinsic activities were significantly lower. NA was more potent than phenylephrine in these vessels, and there was no significant difference in intrinsic activity. The results suggest that in human omental arteries, the postjunctional α -adrenoceptors are mainly of the α_1 -type, even if a small population of α_2 -adrenoceptors cannot be excluded. In omental veins, there seems to be a functionally important population of postjunctional α_2 -adrenoceptors occurring together with a population of α_1 -adrenoceptors.

Key words: Human omental vessels, α -adrenoceptor subtypes, arteries, veins, α_1 -adrenoceptors, α_2 -adrenoceptors

It is generally accepted that α -adrenoceptors (α -receptors) should be classified pharmacologically rather than according to anatomical localization (Berthelsen & Pettinger 1977, Wikberg 1978, Starke & Langer 1979). The α_1 -receptors are those that, like the postjunctional α -receptors of the rabbit pulmonary artery, are sensitive to low concentrations of, e.g., phenylephrine and prazosin, whereas α_2 -receptors are those that, like the prejunctional α_2 -receptors of the rabbit pulmonary artery, are sensitive to low concentrations of, e.g., clonidine, oxymetazoline, and rauwolscine (for reviews see Langer & Shepperson 1981, Starke 1981).

Both subtypes of α -receptor have been identified postjunctionally in vascular smooth muscle (for reviews see Timmermans & Van Zwieten 1981,

McGrath 1982, Van Meel 1982), but the variation between species (Ruffolo et al. 1982) and different vascular regions (Ruffolo et al. 1981, Horn et al. 1982) seems to be pronounced. The information on vascular α -receptor subgroups in human tissue is scarce. Stevens & Moulds (1981, 1982) reported that human digital arteries and metacarpal veins obtained post mortem were sensitive to both α_1 - and α_2 -receptor selective substances. Supporting this finding, it has been found that human forearm blood flow was sensitive both to drugs with preference for α_1 -receptors (Amann et al. 1980) and to drugs with selective action on α_2 -receptors (Van Brummelen et al. 1982).

The present study was performed in order to characterize and compare the postjunctional α -re-

ceptor populations in human omental arteries and veins obtained during surgery.

MATERIAL AND METHODS

Preparation and mounting

Branches of the gastroepiploic vessels were taken from fifteen adult normotensive patients (8 women and 7 men, age 18 to 77, mean 54 years) undergoing laparotomy because of gall stones (8), peptic ulcer (3), hiatic hernia (1), appendicitis (1) and abdominal aortic aneurysm (2). Only patients free from drug treatment were chosen. The operations were all done under general anaesthesia: atropine, diazepam, thiopental sodium, suxamethonium, fentanyl, pancuronium, and ventilation with a gas mixture of 60% N₂O and 40% O₂.

Parts of the omentum were taken out, placed in chilled Krebs-Ringer solution (for composition see below) and immediately transported to the laboratory for further dissection. The main artery and concomitant vein in the specimen (outer diameter *in situ* 0.4–0.6 mm and 1.0–1.8 mm, respectively) were dissected free from the omental fat under an operation microscope and care was taken to avoid stretching or other types of injury of the vessels. From each artery and vein, six vessel segments, 1–3 mm long, were used. Some preparations were immediately mounted in temperature-controlled organ baths, whereas the rest was kept in Krebs-Ringer (refrigerator) for later use (within 24 h). The Krebs-Ringer solution had the following composition (in mM): NaCl 119, NaHCO₃ 15, KCl 4.6, CaCl₂ 1.5, NaH₂PO₄ 1.2, MgCl₂ 1.2 and glucose 11. Cocaine, 10⁻⁶ M (for blockade of neuronal uptake), and propranolol, 10⁻⁷ M (to block beta-adrenoceptors), were also added. In the baths, the buffer solution was continuously bubbled with a mixture of 5% CO₂ and 95% O₂ giving a pH of approximately 7.4. The temperature was maintained at 37°C.

The vessel segments were suspended between two L-shaped metal holders (0.2 mm in diameter), one of which was attached to a Grass FT 03 transducer connected to a Grass polygraph for continuous recording of isometric tension. The position of the other holder could be changed by means of a movable unit allowing fine adjustments of vessel tension by varying the distance between the metal prongs (for further experimental details see Högestätt, Andersson & Edvinsson 1983). A tension of about 6 mN was applied to the vessels resulting in a spontaneous relaxation. This was compensated for by frequent stretches in order to maintain a tension of approximately 4 mN. In separate experiments it was ascertained that an optimum contractile response was obtained at this basal tension. After approximately one hour, when a steady level of 4 mN had been obtained, the vessels were contracted by exposure to a K⁺-rich buffer solution containing (in mM): KCl 124, NaHCO₃ 15, CaCl₂ 1.5, NaH₂PO₄ 1.2, MgCl₂ 1.2, glucose 11.

After two reproducible contractions (variation less than 10%) had been evoked by the K⁺-rich Krebs solution an agonist was added cumulatively and concentration-response (cr) curves were constructed on basis of the data obtained. In some of the experiments, one or two cr-

curves were performed with noradrenaline before any other agonist was tested.

The antagonist experiments were performed by studying five segments from the same artery or vein simultaneously. After two reproducible K⁺-contractions had been obtained, three segments were exposed to various antagonist concentrations at least 15 min prior to the cumulative addition of noradrenaline, whereas two segments served as controls. The mean EC₅₀-value (i.e. the agonist concentration at which 50% of maximum contraction occurs) for noradrenaline in the two control segments served as reference to which the EC₅₀-values of the other segments in the same experiment were compared when calculating the concentration ratios for the pA₂-determinations (see below).

The K⁺-induced contraction was used as an internal standard in each segment, and each response was expressed as a percentage of the K⁺-induced contraction. In separate experiments it was secured that pretreatment with phenolamine (10⁻⁶ M) reduced the K⁺-induced contraction by less than 10%. This was valid for both arteries and veins. In the arteries the amplitude of contraction was 17.0 ± 1.9 mN and in the veins 9.8 ± 1.4 mN (n = 15). It was also shown in preliminary experiments that an increase in cocaine or propranolol concentrations beyond those used in this investigation did not potentiate the NA response.

Analysis of data

pA₂, the negative logarithm of the antagonist concentration that displaces the agonist cr-curves towards higher concentrations with a factor of 2, was determined according to Arunlakshana & Schild (1959). According to Van den Brink (1977) it is possible to calculate pA₂-values even if the antagonist, besides shifting the cr-curve, also reduces maximum contraction. By calculating the concentration ratio from the EC₅₀-values in each individual curve, the shift of the curve is estimated from responses with relatively equal effects.

All cr-curves were plotted graphically and E_{max}, i.e., the maximum contraction obtained with an agonist, and pD₂, i.e., the negative logarithm of the EC₅₀-value, were calculated. Due to difficulties in calculating adequate pD₂-values for cr-curves with low E_{max}, only cr-curves with E_{max} exceeding 10% of the K⁺-induced contractions were included in the results.

Statistics

Unpaired Student's *t*-test was used to determine statistical significance. The regressions of the Schild plots, based on linear least square fit, and the *t*-test analyses in conjunction with these, were performed on a calculator (Hewlett Packard 41 CV) using a program kindly supplied by Dr Claus Rerup, Department of Pharmacology, University of Lund, Lund. The standard deviation of the single measurement was calculated from the differences in the duplicates and derived as $s = \pm \sqrt{(d^2/2n)}$ where *s* is the standard deviation, *d* the difference between duplicates and *n* the number of double determinations.

Drugs

± Noradrenaline hydrochloride (Sigma), L-phenylephrine hydrochloride (Sigma), oxymetazoline chloride (Draco),

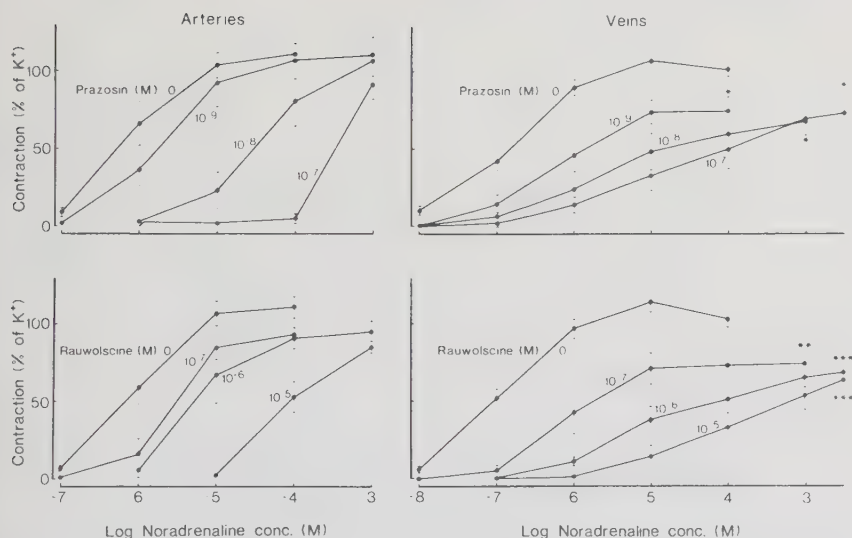


Fig. 1. Concentration-response curves for NA in the absence and presence of increasing concentrations of prazosin (upper panel) and rauwolscline (lower panel). Arteries are shown to the left, and veins to the right. The contractile responses are expressed as percentage of the K^+ (124 mM)-induced contraction. Significant reductions in the maximum response to NA are denoted: * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$). Each point in the curves indicates mean $\pm$ standard error and results obtained from 6 patients.

clonidine hydrochloride (ACO), prazosin hydrochloride (Pfizer), rauwolscline hydrochloride (Roth), cocaine hydrochloride (ACO), propranolol hydrochloride (ICI).

The drugs were dissolved in 0.9% NaCl containing 1.0 mM ascorbic acid except prazosin which in the concentration 1 mM was dissolved in 10% lactic acid. Stock solutions of the drugs were prepared and stored at -70°C until use. Fresh dilutions (in 0.9% NaCl and 1.0 mM ascorbic acid) were prepared daily. The concentrations in results are given as final molar concentrations in the organ baths.

RESULTS

Effects of α -receptor antagonists

Arteries. Prazosin caused a displacement of the NA cr-curves to the right without significant reduction of E_{max} (Fig. 1). In the presence of prazosin 10 M no plateau was reached even with the highest NA-concentration used (10^{-3} M). When calculating the EC_{50} -value at this concentration of prazosin

Table 1. Data on the Schild regressions

NA was used as agonist. The values represent mean and the 95% confidence interval. Each regression line was determined from 17–18 values. ** ($p < 0.01$) and *** ($p < 0.001$) denote level of significance of the correlation. Values within brackets are the 95% confidence intervals

Omental vessel	Antagonist	Slope of regression line	Correlation coefficient	pA_2 -value
Artery	Prazosin	1.08 (0.88–1.29)	0.95***	9.48 (9.20–9.89)
Artery	Rauwolscline	0.76 (0.47–1.05)	0.82***	7.19 (6.76–8.03)
Vein	Prazosin	0.76 (0.32–1.20)	0.68**	9.72 (8.99–12.19)
Vein	Rauwolscline	0.94 (0.58–1.30)	0.81***	8.11 (7.47–9.49)

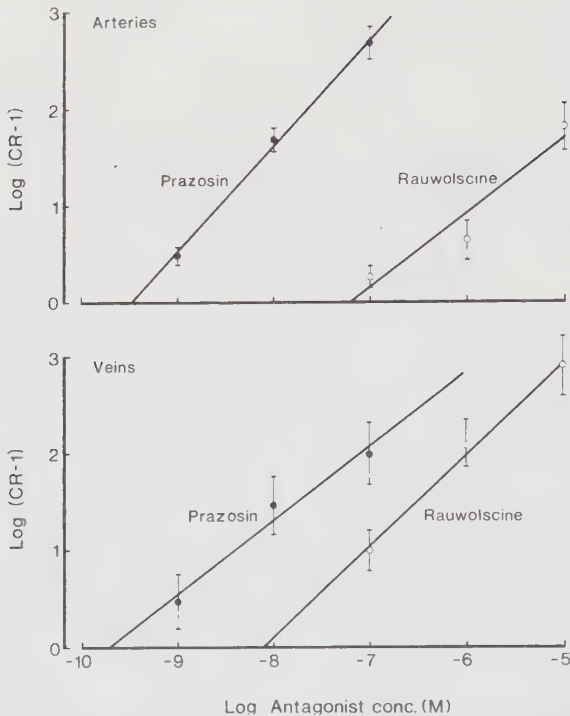


Fig. 2. Effects of prazosin (●) and rauwolscine (○) on responses of human omental vessels. The log [concentration ratio (CR)–1] is plotted vs the log antagonist concentration in omental arteries and veins. Each regression is calculated from vessels of 6 patients and each point denotes mean $\pm$ standard error from these.

it was assumed that no depression of $E_{\max}$ had occurred. When the interaction between NA and prazosin was illustrated by a Schild plot (see Methods), a regression line was obtained with a slope of 1.08 and a pA_2 -value of 9.48 (Fig. 2, Table 1).

Rauwolscine also shifted the NA cr-curve towards higher concentrations. $E_{\max}$ was not significantly reduced by 10^{-7} and 10^{-6} M of the antagonist, whereas no plateau of the NA cr-curve was observed with 10^{-5} M rauwolscine (Fig. 1). At this concentration of rauwolscine, the EC_{50} -value for NA was calculated assuming $E_{\max}$ to be unchanged. The slope of the Schild plot was 0.76 which did not significantly differ from unity. The pA_2 -value was 7.19 (Fig. 2, Table 1).

Veins. Both prazosin and rauwolscine displaced the cr-curve for NA towards higher concentrations and both antagonists significantly depressed $E_{\max}$ (Fig. 1). Concentration ratio was calculated according to Van den Brink (1977) (see Methods) and the relation between log (CR–1) and the negative logarithm of the antagonist concentration was illustrated in Schild plots despite the reduction of $E_{\max}$. The slopes were 0.94 and 0.76 and the pA_2 -values 8.11 and 9.72 for rauwolscine and prazosin, respectively (Fig. 2, Table 1).

Intra- and interindividual variation of pD_2 -values

The reproducibility of the method was estimated by comparing the pD_2 -values for NA from two vein

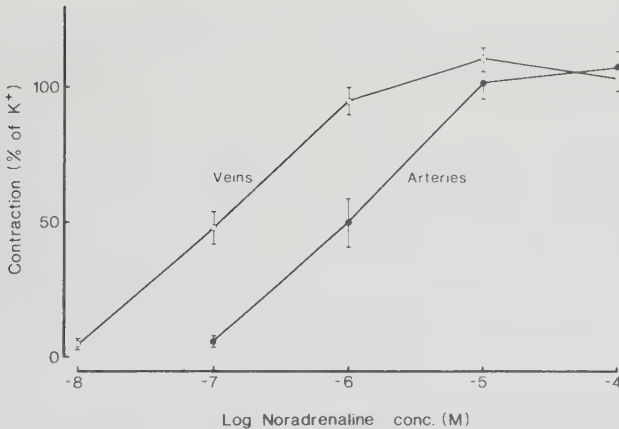


Fig. 3. Concentration-response curves for NA in human omental arteries (●) and veins (○) expressed as percentage of the K^+ (124 mM)-induced contraction. Each point is the mean $\pm$ standard error of segments from 8 patients.

segments from each of 8 patients (see Methods). The coefficient of variation, obtained by dividing s with the mean pD_2 -value, was 3%.

As an estimation of interindividual variation, the mean NA- pD_2 -values for two vein segments from each of the 8 patients were added and mean $\pm s$ were determined giving a coefficient of variation of 5%. The coefficient of variation was 4% and 6% in arterial segments for the intra- and interindividual variation, respectively.

Effects of α -receptor agonists

NA was significantly ($p < 0.001$) more potent in the veins than in the arteries (Fig. 3), whereas the intrinsic activity related to the K^+ -induced contraction was similar in the two preparations.

Arteries. Clonidine in the concentrations 10^{-8} – 10^{-4} M caused no contraction in any of the arteries from six patients examined. Arterial segments from only one of six patients displayed contractions upon exposure to oxymetazoline. The mean cr-curve for these preparations is depicted in Fig. 4.

Phenylephrine concentration-dependently contracted all segments tested. NA was significantly more potent than phenylephrine ($p < 0.005$), but both drugs had similar intrinsic activity (Fig. 4, Table II).

Veins. Veins from three of seven patients contracted concentration-dependently upon exposure to clonidine. Oxymetazoline contracted veins from four out of six patients. Neither clonidine nor oxy-

Table II. Effects of α -adrenoceptor agonists on isolated human omental arteries and veins

Values represent mean and n denotes number of patients. Values within brackets are the 95% confidence intervals

Agonist	n	Arteries		n	Veins	
		pD_2	E_{max} (% of K^+)		pD_2	E_{max} (% of K^+)
Phenylephrine	6	5.62 (5.11–6.13)	100 (64–136)	6	5.65 (5.01–6.29)	80 (54–106)
Clonidine	6	–	<10	3	6.81 (5.65–7.97)	18 (0–44)
Oxymetazoline	1	7.14	31	4	6.74 (6.27–7.21)	47 (5–89)
Noradrenaline	14	6.07 (5.88–6.26)	114 (99–129)	14	6.93 (6.69–7.17)	104 (93–115)

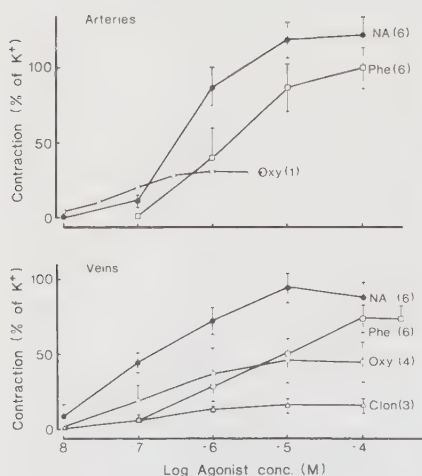


Fig. 4. Concentration-response curves for noradrenaline (NA, ●), clonidine (Clon, △), oxymetazoline (Oxy, ○), and phenylephrine (Phe, □) in human omental arteries and veins. The effects of all drugs were examined in vessels from six patients (except clonidine which was examined in veins from seven patients). Numbers within brackets denote number of patients with responding vessels. Each point indicates mean $\pm$ standard error (except data on oxymetazoline in the arteries and is expressed as percentage of the K^+ (124 mM)-induced contraction).

metazoline had pD_2 -values significantly different from that of NA (Table II). However, both drugs had E_{max} -values that were significantly lower than that for NA ($p < 0.02$ and $p < 0.001$ for oxymetazoline and clonidine, respectively).

NA was more potent than phenylephrine ($p < 0.001$, Table II). The difference in intrinsic activity was, however, not significant.

DISCUSSION

The occurrence of differences between the α -adrenoceptor populations in arteries and veins have been suggested by several investigators. Thus, the postjunctional α -receptors in canine arteries were shown to be mainly of α_1 -type, whereas in veins both α_1 - and α_2 -receptors appeared to mediate contraction (De Mey & Vanhoutte 1980, 1981). The vascular α -receptors in human digital arteries and metacarpal veins displayed unusual pharmacological characteristics (Stevens & Moulds 1981, 1982),

which were explained either by the presence of different proportions of α_1 - and α_2 -receptors in these vessels or by the presence of α -receptors different from "classical" α_1 - and α_2 -receptors. In the human nasal mucosa, arterioles controlling blood flow were suggested to contain mainly α_2 -receptors, as only selective α_2 -receptor agonists reduced mucosal blood flow and the responses were blocked by yohimbine (Andersson & Bende 1983). However, both α_1 - and α_2 -receptors seem to occur in the capacitance vessels as these vessels contract (nasal decongestion) in response to both α_1 - and α_2 -receptor agonists (Andersson & Bende 1983). Thus, the characteristics of the postjunctional α -receptors appear to exhibit considerable regional variations.

The human omental arteries of the present study appeared to be less sensitive to NA than reported previously by Alkjaer & Mulvany (1981) (pD_2 6.08 vs 6.43). However, this discrepancy may be attributed to differences in experimental technique. In the human omental arteries of the present study clonidine was without effect, and in arterial segments from only one of six patients oxymetazoline was able to elicit a response exceeding 10% of the K^+ -evoked contraction. These results differ from those reported by Stevens & Moulds (1982) in isolated human digital arteries. They found that E_{max} for oxymetazoline was 80%, and that of clonidine 55% of the contraction evoked by 80 mM K^+ . Thus, the present data do not favour the occurrence of a functionally important α_2 -receptor function in human omental arteries.

The relative potency of phenylephrine against NA (0.17) in the present study is similar to the values for the postjunctional α_1 -receptor found in guinea-pig aorta and rabbit pulmonary artery (0.12 and 0.23, see Wikberg 1979). Stevens & Moulds (1982) found a relative potency of 0.66 for phenylephrine in human digital arteries. Taken together these data suggest a predominance of α_1 -receptors in human omental arteries. This suggestion is further supported by the competitive interaction of prazosin with NA in these vessels. The Schild plot had a slope of 1.08 and the pA_{21} -value was 9.48. The pA_2 values of prazosin for interaction with α_1 receptors have been reported to range between 7.9 and 8.9 in several vessels, such as canine splenic and femoral arteries (De Mey & Vanhoutte 1981), rabbit portal vein (Docherty & Starke 1981), rabbit aorta (Cavero et al. 1978, Docherty & Starke 1981),

rabbit pulmonary artery (Docherty & Starke 1981), and cat lingual arteries (Skårby et al. 1983). Only in rat mesenteric arteries (Cohen et al. 1980) the pA_2 values of prazosin (9.89) appeared to be higher than in human omental arteries and veins.

Even though oxymetazoline and clonidine had little or no contractile effect in the arteries, rauwolscine was more potent than described in other preparations where the receptors are considered to be solely of α_1 -type. The pA_2 -values in rabbit pulmonary artery (Weitzell et al. 1979, Docherty & Starke 1981), rabbit aorta and portal vein (Docherty & Starke 1981) were all between 5.3–5.9 which is considerably lower than the value of 7.19 found in human omental arteries. Thus, it cannot be excluded that rauwolscine also interacts with a population of α_2 -receptors in these arteries. If rauwolscine interacts with two different types of receptor, a slope lower than unity would be expected. However, the slope of the Schild plot was found to be 0.76, which was not significantly different from one. Another possibility is that the α_1 -receptors of human omental arteries have an unusually high sensitivity to rauwolscine, which supports the view of Stevens & Moulds (1982) that the postsynaptic α_1 -receptors in human vascular smooth muscle are atypical.

The veins from most patients responded to both clonidine and oxymetazoline, and the relative potency of clonidine when compared to NA (1.15) was close to the value obtained for prejunctional α -receptors in the rabbit pulmonary artery (1.2) (Starke et al. 1975). This may indicate the presence of a population of postjunctional α_2 -receptors in the human veins. Both rauwolscine and prazosin reduced significantly the maximum response to NA. Stevens & Moulds (1982) also found prazosin to significantly depress E_{max} in human metacarpal veins. These findings suggest a non-competitive interaction of these drugs with the α -receptor. Van den Brink (1977) suggested that pA_2 -values could still be calculated accurately if the reduction in maximum response to NA was compensated for (see Methods). With this type of calculation, a slope of 0.94 and pA_2 -value of 8.11 were obtained with rauwolscine. These findings also favour the presence of α_2 -receptors in human omental veins. It is, however, noteworthy that rauwolscine was a potent antagonist even when the response to clonidine was absent, a finding that could be expected if the two drugs did not interact with the same site.

The high pA_2 -value of prazosin and the pD_2 -

value of phenylephrine suggest a population of postjunctional α_1 -receptors in human veins.

The potency difference between arteries and veins was similar for noradrenaline and rauwolscine (9.3 and 8.3 times, respectively), and phenylephrine and prazosin (1.1 and 1.7 times, respectively) were similarly potent in the two vascular beds. It may therefore be speculated that the higher potency of NA in veins is mainly due to a larger population of α_2 -receptors in these vessels.

Based on the widely accepted pharmacological classification of α -receptors (Wikberg 1978, Starke & Langer 1979), the present results thus suggest that in human omental arteries the postjunctional α -receptors are mainly of α_1 -type, even though a small population of α_2 -receptors cannot be excluded. On the other hand, it is conceivable that in omental veins there is a functionally important population of postjunctional α_2 -receptors occurring together with a population of α_1 -receptors. If the present *in vitro* data can be extrapolated to the *in vivo* situation, these findings suggest that it should be possible to selectively influence the arterial and venous sides of this vascular bed.

REFERENCES

- ALKJAER, C. & MULVANY, M. J. 1981. Functional and morphological properties of human omental resistance vessels. *Blood Vessels* 18: 233–244.
- AMANN, F. W., BOLLI, P., KOWSKI, W. & BÜHLER, F. R. 1980. Verstärkte α -Adrenozeptor-vermittelte Vasokonstriktion bei essentieller Hypertonie. *Schweiz Med Wochenschr* 110: 1941–1944.
- ANDERSSON, K.-E. & BENDE, M. 1983. The role of adrenoceptors in the control of human nasal mucosal blood flow. *Ann Otol Rhinol Laryngol* (in press).
- ARUNLAKSHANA, O. & SCHILD, H. O. 1959. Some quantitative uses of drug antagonists. *Br J Pharmacol* 14: 48–59.
- BERTHESEN, S. & PETTINGER, W. A. 1977. A functional basis for classification of α -adrenergic receptors. *Life Sci* 21: 595–606.
- CAVERO, I., FENARD, S., GOMENI, G., LEFEVRE, F. & ROACH, A. G. 1978. Studies on the mechanisms of the vasodilator effects of prazosin in dogs and rabbits. *Eur J Pharmacol* 49: 259–270.
- COHEN, M. L., WILEY, K. S. & LANDRY, A. S. 1980. *In vitro* comparison of the pre- and postsynaptic alpha adrenergic receptor blocking properties of prazosin and tiodazosin (BL 5111). *Clin Exp Hypertens* 2: 1067–1082.
- DE MEY, J. G. & VANHOUTTE, P. M. 1980. Differences in pharmacological properties of postjunctional alpha-adrenergic receptors among arteries and veins. *Arch Int Pharmacodyn* 244: 328–329.

- DE MEY, J. & VANHOUTTE, P. M. 1981. Uneven distribution of postjunctional α_1 - and α_2 -like adrenoceptors in canine arterial and venous smooth muscle. *Circ Res* 48: 875-884.
- DOCHERTY, J. R. & STARKE, K. 1981. Postsynaptic α -adrenoceptor subtypes in rabbit blood vessels and rat anococcygeous muscle studied in vitro. *J Cardiovasc Pharmacol* 3: 854-866.
- HÖGESTÄTT, E. D., ANDERSSÖN, K.-E. & EDVINSÖN, L. 1983. Mechanical properties of rat cerebral arteries as studied by a sensitive device for recording of mechanical activity in isolated small blood vessels. *Acta Physiol Scand* 117: 49-61.
- HORN, P. T., KOHLI, J. D., LISTINSKY, J. J. & GOLDBERG, L. I. 1982. Regional variation in α adrenergic receptors in the canine resistance vessels. *Naunyn-Schmiedeberg's Arch Pharmacol* 318: 116-172.
- LANGER, S. Z. & SHEPPERSON, N. B. 1981. Subclassification of α -adrenoceptors into α_1 - and α_2 -categories: relevance to antihypertensive therapy. In: *New trends in arterial hypertension. Inserm Symposium No. 17* (ed. M. Worcel et al.), pp. 73-85. Elsevier/North Holland Biomedical Press.
- MCGRATH, J. C. 1982. Evidence for more than one type of postjunctional α -adrenoceptor. *Biochem Pharmacol* 31: 467-484.
- RUFFOLO, R. R., WADDELL, J. E. & YADEN, E. L. 1981. Postsynaptic α -adrenergic receptor subtypes differentiated by yohimbine in tissues from the rat. Existence of α_2 -adrenergic receptors in rat aorta. *J Pharmacol Exp Ther* 217: 235-240.
- RUFFOLO, R. R., WADDELL, J. E. & YADEN, E. L. 1982. Heterogeneity of postsynaptic α adrenergic receptors in mammalian aortas. *J Pharmacol Exp Ther* 221: 309-314.
- SKARBY, T. V. C., ANDERSSÖN, K.-E. & EDVINSÖN, L. 1983. Pharmacological characterization of postjunctional α -adrenoceptors in isolated feline cerebral and peripheral arteries. *Acta Physiol Scand* 117: 63-73.
- STARKE, K. 1981. α -Adrenoceptor subclassification. *Rev Physiol Biochem Pharmacol* 88: 199-236.
- STARKE, K. & LANGER, S. Z. 1979. A note on terminology for presynaptic receptors. In: *Presynaptic receptors* (ed. S. Z. Langer, K. Starke & M. L. Dubocovich), pp. 1-3. Pergamon Press, Oxford.
- STARKE, K., ENDO, T. & TAUBE, H. D. 1975. Relative pre- and postsynaptic potencies of α -adrenoceptor agonists in the rabbit pulmonary artery. *Naunyn-Schmiedeberg's Arch Pharmacol* 291: 55-78.
- STEVENS, M. J. & MOULDS, R. F. W. 1981. Heterogeneity of postjunctional α -adrenoceptors in human vascular smooth muscle. *Arch Int Pharmacodyn* 254: 43-57.
- STEVENS, M. J. & MOULDS, R. F. W. 1982. Are the pre- and postsynaptic α -adrenoceptors in human vascular smooth muscle atypical? *J Cardiovasc Pharmacol* 4: S129-S133.
- TIMMERMANS, P. B. M. W. M. & VAN ZWIETEN, P. A. 1981. The postsynaptic α_2 -adrenoceptor. *J Auton Pharmacol* 1: 171-183.
- VAN BRUMMELEN, P., VERMEY, P., TIMMERMANS, P. B. M. W. M. & VAN ZWIETEN, P. A. 1982. Preliminary evidence for a postsynaptic α_2 -adrenoceptor in the vasculature of the human forearm. *Proceedings of the B. P. S., 8th-10th September 1982*, pp. 134P-135P.
- VAN DEN BRINK, F. G. 1977. General theory of drug-receptor interactions. In: *Kinetics of drug action* (ed. Van Rossum), pp. 247-249. Springer, Heidelberg and New York.
- VAN MEEL, J. C. A. 1982. The vascular postjunctional α_2 -adrenoceptor. *Rodopi Amsterdam* (Thesis).
- WEITZELL, R., TANAKA, T. & STARKE, K. 1979. Pre- and postsynaptic effects of yohimbine stereoisomers on noradrenergic transmission in the pulmonary artery of the rabbit. *Naunyn-Schmiedeberg's Arch Pharmacol* 308: 127-136.
- WIKBERG, J. 1978. Differentiation between pre- and postjunctional α -receptors in guinea-pig ileum and rabbit aorta. *Acta Physiol Scand* 103: 225-239.
- WIKBERG, J. E. S. 1979. The pharmacological classification of adrenergic α_1 and α_2 receptors and their mechanism of action. *Acta Physiol Scand Suppl.* 468.

**POSTJUNCTIONAL α_1 - AND α_2 -ADRENOCEPTORS MEDIATING CONTRACTION IN
ISOLATED HUMAN GROIN ARTERIES AND VEINS**

Stig Steen, Trygve Sjöberg, Tor V.C. Skärby,
Lars Norgren and Karl-Erik Andersson

Departments of Clinical Pharmacology and Surgery,
University of Lund, Lund, Sweden

Please send correspondence to:

Stig Steen, M.D.
Department of Surgery
University of Lund
S-221 85 LUND
Sweden

STEEN, S., SJÖBERG, T., SKÄRBY, T.V.C., NORRGREN, L. & ANDERSSON, K.-E.

Postjunctional α_1 - and α_2 -adrenoceptors mediating contraction in isolated human groin arteries and veins. Acta Physiol. Scand.

By means of selective agonists and antagonists for α_1 - and α_2 -receptors, the α -receptor subtypes in human groin arteries and veins were characterized and compared. In the arteries the α_1 -receptor blocker prazosin caused a concentration-dependent parallel displacement of the noradrenaline (NA) concentration-response (cr) curve without reduction of maximum ($pA_2 = 9.86$); the selective α_2 -receptor antagonist rauwolscine in the concentration 10^{-8} M caused a right-ward shift of the NA cr-curve without reduction of E_{max} , but 10^{-7} M and 10^{-6} M caused little or no further shift. In the veins, the two antagonists had the opposite effects. Rauwolscine caused a concentration-dependent right-ward shift of the NA cr-curve without depression of maximum ($pA_2 = 9.03$); prazosin 10^{-9} M significantly displaced the NA cr-curve, whereas 10^{-8} M and 10^{-7} M caused little or no further shift. The responses to the α_2 -receptor agonist clonidine in the arteries were too small to allow calculations of pEC_{50} values, in the veins contractions were elicited in all vessel segments investigated ($pEC_{50} = 6.24$). Phenylephrine, selective for α_1 -receptors was significantly more potent in arteries than in veins. NA was significantly more potent in veins than in arteries. It is concluded that in human groin vessels, there is a functional predominance of α_1 -receptors in the arteries and of α_2 -receptors in the veins.

Key words: Human groin vessels, α -adrenoceptor subtypes, arteries, veins, α_1 -adrenoceptors, α_2 -adrenoceptors.

INTRODUCTION

It is now generally accepted that α -adrenoceptors (α -receptors) may be classified according to their pharmacological characteristics (Berthelsen & Pettinger 1977, Wikberg 1978, Starke & Langer 1979). Using this mode of classification, results have been obtained suggesting that both α_1 - and α_2 -receptors can mediate contraction in vascular smooth muscle (for reviews see Timmermans & Van Zwieten 1981, McGrath 1982, Van Meel 1982, Ruffolo 1983). The vascular postjunctional α -receptor subtype has been shown to vary depending on the species (Ruffolo et al. 1982) and the region studied (Ruffolo et al. 1981, Horn et al. 1982, Skärby et al. 1983).

To correct tissue defects of trauma patients, a free flap transfer involving microvascular anastomoses is often used. The free flap is usually taken from the groin, raised on the superficial circumflex iliac vessels (Ohmori & Harii 1979). By incorporating also the superficial epigastric vessels within the groin flap, an enormous area of skin can be transplanted freely to any area of the body (Taylor & Silber 1979) (Fig 1). In connection with surgery, α -receptor blockade has been recommended to achieve the best possible blood flow through the free flap (Robins 1983). Information of the populations of α -receptors in vessels from the groin region may therefore be of particular interest. The present study was performed in order to obtain such information and to characterize and compare the postjunctional α -receptor populations in human groin veins and arteries.

MATERIAL AND METHODS

Twenty patients (12 women and 8 men, aged 23 to 71 years, mean 47 years) undergoing surgery because of primary varicose veins were chosen as vessel donors. All of them were healthy and normotensive and without drug treatment. Twelve operations were performed under epidural anesthesia and 8 were carried out under thiopental-halothane anesthesia. The groin dissection was done with special care not to damage the small tributaries of the terminal segment of the long saphenous vein ("stella venosum", see Fig. 1). Segments of macroscopically normal veins, 1-2 mm in outer diameter in situ, were dissected free and removed, and care was taken to avoid stretching or other type of injury. The superficial external pudendal artery (see Fig. 1) was carefully dissected free from the end of the long saphenous vein and followed medially for 2 - 3 cm and then extirpated between hemostats. The removed segment had an outer diameter in situ of 0.5 - 1.2 mm. All vessel segments were immediately placed in chilled Krebs-Ringer solution. Under an operation microscope the vessels were dissected free from fat, cut into segments of approximately 1 mm length and mounted in Krebs-Ringer organ baths (37°C). Cocaine, 10^{-6} M (for blockade of neuronal uptake), and propranolol, 10^{-7} M (to block beta-adrenoceptors), were always present in the baths, which were continuously bubbled with a mixture of 5 % CO₂ and 95 % O₂ giving a pH of approximately 7.4.

The vessel segments were suspended between two L-shaped metal holders (0.2 mm in diameter), one of which was attached to a Grass FT 03 transducer connected to a Grass polygraph for continuous recording of isometric tension (for experimental details, see Högestätt et al. 1983). In the experiments on agonists the vessels were stretched to a passive tension of 4 mN. During these experiments the number of "non-responding" vessels was high. Separate experiments showed that a passive tension of 8 - 12 mN produced a maximum contractile response to K⁺ in both arteries and

veins. Therefore in the subsequent (antagonist) experiments an initial tension of 8 - 12 mN was applied to the vessels. Using this approach practically all vessels responded to the drugs used. The increase in initial tension did not significantly alter the potency of noradrenaline (NA) or the maximum contractile response when expressed as percentage of the K^+ -induced contraction. When a steady tension level had been obtained (after 1 - 3 hours), the vessels were contracted by exposure to a K^+ -rich buffer solution containing (in mM): KCl 124, $NaHCO_3$ 15, $CaCl_2$ 1.5, NaH_2PO_4 1.2, $MgCl_2$ 1.2, glucose 11.

Investigations

After two reproducible contractions (accepted variation less than 10 %) had been evoked by the K^+ -rich Krebs solution, an agonist was added cumulatively and concentration-response (cr) curves were constructed on basis of the data obtained.

The experiments on antagonists were performed by studying 3 arterial and 3 vein segments from the same patient simultaneously. After performing NA cr-curves in all vessels, one arterial and one vein segment were exposed to prazosin whereas another arterial and vein segment were exposed to rauwolscine. The reproducibility of the NA cr-curves was examined in parallel in the third arterial and vein segment. If tachyphylaxis was observed in these control curves, the experiment was excluded. The antagonists were in contact with the tissue for at least 15 min prior to the cumulative addition of NA. After NA cr-curves had been obtained, all vessels segments were washed in Krebs-Ringer solution for a minimum of 45 min. The same procedure was hereafter repeated twice, but now with ten respectively hundred times higher antagonist concentrations.

Each response was expressed in percentage of the K^+ -induced contraction. In separate experiments it was secured that pretreatment with phentolamine (10^{-6} M) reduced the K^+ -induced contraction by less than 10 %. This was valid for both arteries and veins. In the arteries the amplitude of contraction was $21.2 \pm$

3.1 mN and in the veins 29.5 ± 4.9 mN ($n=20$). It was also shown in preliminary experiments that an increase in cocaine or propranolol concentrations beyond those used in this investigation did not potentiate the NA response.

There were no differences in reaction to drugs that could be related to the type of anesthesia or to the sex of the patients.

Analysis of data

All cr-curves were plotted graphically and $E_{\max}$, i.e. the maximum contraction obtained with an agonist, and EC_{50} , i.e. the agonist concentration at which 50 % of maximum effect occurs, were calculated. pEC_{50} is defined as the negative logarithm of the EC_{50} value. pA_2 , i.e. the negative logarithm of the antagonist concentration that displaces the agonist cr-curves towards higher concentrations with a factor of 2, was determined according to the method of Arunlakshana & Schild (1959) (Schild plot). The concentration ratio (CR) was calculated as the ratio between the EC_{50} -values in presence and in absence of antagonist in two parallel vessel segments.

Statistics

Unpaired Student's t -test was used to determine statistical significance. The regressions of the Schild plots, based on linear least square fit, were performed on a calculator (Hewlett Packard 41 CV) using a program kindly supplied by Dr Claus Rerup, Department of Pharmacology, University of Lund, Lund.

Drugs

d1-Noradrenaline hydrochloride (Sigma), 1-phenylephrine hydrochloride (Sigma), oxymetazoline chloride (Draco), clonidine hydrochloride (ACO), prazosin hydrochloride (Pfizer), rauwolscine hydrochloride (Roth), cocaine hydrochloride (ACO) and propranolol hydrochloride (ICI).

The drugs were dissolved in 0.9 % NaCl containing 1.0 mM ascorbic acid except prazosin which in the concentration 1 mM was dissolved in 10 % lactic acid. Stock solutions of the drugs were prepared and stored at -70 °C until use. Fresh dilutions (in 0.9 % NaCl and 1.0 mM ascorbic acid) were prepared daily. The concentrations are given as final molar concentrations in the organ baths.

RESULTS

Effects of α -receptor antagonists

There was no tachyphylaxis in the NA cr-curves performed as controls in parallel with the antagonist experiments, except in vessels from two patients. All data from these patients were therefore excluded.

Arteries. Prazosin caused a concentration-dependent parallel right-ward displacement of the NA cr-curve without significant reduction of $E_{\max}$ (Fig. 2). A Schild plot of the data gave a slope of 0.94 and a pA_2 value of 9.86 (Table I).

Rauwolscine 10^{-8} M caused a shift of the NA cr-curve to the right (Fig. 2). 10 and 100 times higher concentrations caused little or no further shift and no Schild plot was therefore calculated. No significant depression of $E_{\max}$ was observed.

Veins. Prazosin 10^{-9} M significantly displaced the NA cr-curve, whereas 10^{-8} M and 10^{-7} M caused little or no further shift (Fig. 2). A pA_2 -value could therefore not be calculated. Rauwolscine caused a concentration dependent parallel shift of the NA cr-curve (Fig. 2). The slope of the Schild plot was not significantly different from unity. The pA_2 value was 9.03 (Table 1). The antagonists did not significantly attenuate $E_{\max}$.

Effects of α -receptor agonists

The effects of the agonists used are illustrated in Fig. 3, and the EC_{50} and $E_{\max}$ values given in Table II. The responses elicited by clonidine in the arteries were too small for calculation of EC_{50} -values. In the veins clonidine was able to elicit contractions in all the segments examined.

Phenylephrine was about 12 times more potent ($p < 0.01$), and had higher intrinsic activity ($p < 0.05$) in the arteries than in the veins. The potency and intrinsic activity of oxymetazoline did not differ significantly in the arteries and veins. NA displayed

a higher intrinsic activity in arteries ($p < 0.05$) and appeared to be more potent in veins than in arteries (Table 2). However, the latter difference was only significant ($p < 0.01$) if the pEC_{50} values of the NA controls in the antagonist experiments also were included in the comparison ($pEC_{50} = 6.27$ and 7.00 in arteries and veins respectively, $n = 14$).

DISCUSSION

The pharmacological tools used in the present investigation for characterization of α -receptors are well established. Thus prazosin is one of the most selective α_1 -receptor antagonists at present available, and the selectivity for α_1 -receptors over α_2 -receptors is in the order of 100 - 1000. Rauwolscine, on the other hand, is among the most potent antagonists of α_2 -receptors with selectivity over α_1 -receptors ranging from approximately 30-fold to more than 100-fold. Among the agonists phenylephrine has a selectivity for α_1 -receptors, whereas clonidine preferentially activates α_2 -receptors (see, e.g., Starke 1981, Langer & Shepperson 1981, McGrath 1982, Timmermans & Van Zwieten 1982, Ruffolo 1983).

The slope of the Schild plot of prazosin in the arteries did not differ significantly from unity, which implies that prazosin and NA interact with one and the same type of receptor. The pA_2 -value of prazosin in the arteries (9.86) is in the upper range of pA_2 -values (7.9 - 9.9) found for the interaction between prazosin and α_1 -receptors in a number of different vessels (e.g. Cavero et al. 1978, Cohen et al. 1980, De Mey & Vanhoutte 1981, Docherty & Starke 1981, Skärby et al. 1983, Steen et al. 1984). Phenylephrine had a similar potency and intrinsic activity as NA, whereas clonidine was almost unable to produce contractions. These findings suggest that the contraction mediating α -adrenoceptors in the arteries are of α_1 -type. However, rauwolscine 10^{-8} M also produced a significant shift of the NA cr-curves to the right, but 10 and 100 times higher concentrations of the drug caused no further shift (Fig. 2). This may indicate the presence of a small population of α_2 -receptors.

In the veins rauwolscine caused concentration-dependent shifts of the NA cr-curves. The pA_2 -value, 9.03, was considerably higher than those obtained for the interaction of rauwolscine with α_1 -receptors in rabbit pulmonary artery (Weitzell, Tanaka & Starke 1979, Docherty & Starke 1981), rabbit aorta and portal vein

(Docherty & Starke 1981), which ranged from 5.3 to 5.9. Clonidine was significantly more potent than phenylephrine. These findings indicate that the postjunctional α -receptor is mainly of α_2 -type in the human groin veins. Prazosin 10^{-9} M produced a significant displacement of the NA-cr-curve while little or no further shift was obtained by increasing the prazosin concentration 10 and 100 times. This may indicate the presence of a small population of α_1 -receptors in the veins.

In the present experiments, oxymetazoline, which is considered as a relatively selective α_2 -receptor agonist, elicited conspicuous contractions in the arteries. This is in contrast to the results obtained with clonidine. The discrepancy may be explained by the fact that oxymetazoline in some tissues behaves as a non-selective α -receptor agonist (see Wikberg 1979).

After the completion of the experiments in the present investigation, Glusa & Markwardt (1983) published a study on spirally cut femoral arterial and vein strips from autopsied patients. The authors concluded that these veins seem to contain more α_2 - than α_1 -adrenoceptors postjunctionally whereas in the arteries the α_1 -subtype prevails. Generally, these findings agree well with the results of the present study. However, some discrepancies can be noted; the pA_2 -values of prazosin in the arteries (7.96) and rauwolscine in the veins (7.48) were considerably lower than those of the present study (9.86 and 9.03, respectively). Furthermore, in the study of Glusa & Markwardt (1983) there seemed to be a concentration-dependent shift of the NA cr-curve by rauwolscine in the arteries and by prazosin in veins. These differences may be attributed to differences in the experimental technique and the specimens investigated. E.g., Glusa and Markwardt (1983) used spirally cut strips from large calibered vessels, which were obtained from autopsied patients within 15 h after death.

In a previous study we examined the pharmacological characteristics of the postjunctional α -receptors in human omental arteries and veins with an identical experimental set up as in

the present study (Steen et al. 1984). These results suggested that in human omental arteries the postjunctional α -receptors are mainly of α_1 -type; a small population of α_2 -receptors could not be excluded. Omental veins appeared to be endowed with a functionally important population of postjunctional α_2 -receptors occurring together with a population of α_1 -receptors. These findings are principally the same as for the groin vessels of the present study.

Thus, the present results suggest that in human groin arteries, there is a functional predominance of α_1 -receptors, whereas in corresponding veins, α_2 -receptors predominate. The importance of these findings for the choice of type of α -receptor blockade in connection with a free groin flap transfer, remains to be established.

REFERENCES

- ARUNLAKSHANA, O. & SCHILD, H.O. 1959. Some quantitative uses of drug antagonists. *Br J Pharmacol* 14:48-59.
- BERTHELTSEN, S. & PETTINGER, W.A. 1977. A functional basis for classification of α -adrenergic receptors. *Life Sci* 21:595-606.
- CAVERO, I., FENARD, S., GOMENI, G., LEFEVRE, F. & ROACH, A.G. 1978. Studies on the mechanisms of the vasodilator effects of prazosin in dogs and rabbits. *Eur J Pharmacol* 49:259-270.
- COHEN, M.L., WILEY, K.S. & LANDRY, A.S. 1980. In vitro comparison of the pre- and postsynaptic alpha adrenergic receptor blocking properties of prazosin and tiodazosin (BL 5111). *Clin Exp Hypertens* 2:1067-1082.
- DE MEY, J. & VANHOUTTE, P.M. 1981. Uneven distribution of post-junctional α_1 - and α_2 -like adrenoceptors in canine arterial and venous smooth muscle. *Circ Res* 48:875-884.
- DOCHERTY, J.R. & STARKE, K. 1981. Postsynaptic adrenoceptor subtypes in rabbit blood vessels and rat anococcygeous muscle studied in vitro. *J Cardiovasc Pharmacol* 3:854-866.
- GLUSA, E. & MARKWARDT, F. 1983. Characterisation of post-junctional α -Adrenoceptors in isolated human femoral veins and arteries. *Naunyn Schmiedeberg's Arch Pharmacol* 323:101-105.
- HORN, P.T., KOHLI, J.D., LISTINSKY, J.J. & GOLDBERG, L.I. 1982. Regional variation in alpha adrenergic receptors in the canine resistance vessels. *Naunyn Schmiedeberg's Arch Pharmacol* 318:116-172.
- HÖGESTÄTT, E.D., ANDERSSON, K.-E. & EDVINSSON, L. 1983. Mechanical properties of rat cerebral arteries as studied by a sensitive device for recording of mechanical activity in isolated small blood vessels. *Acta Physiol Scand* 117:49-61.

STARKE, K. & LANGER, S.Z. 1979. A note on terminology for pre-synaptic receptors. In: Presynaptic receptors (ed. S.Z. Langer, K. Starke & M.L. Dubocovich), pp. 1-3. Pergamon Press, Oxford.

STEEN, S., SKÄRBY, T.V.C., NORGREN, L. & ANDERSSON, K.-E. 1984. Pharmacological characterization of postjunctional adrenoceptors in isolated human omental arteries and veins. *Acta Physiol Scand* 120:109-116.

TAYLOR, I. & SILBER, S.J. 1979. Free vascularized transfers of skin flaps, bone, nerve and muscle. In: *Microsurgery*, Williams & Wilkins, Baltimore/London 1979.

TIMMERMANS, P.B.M.W.M. & VAN ZWIETEN, P.A. 1981. The postsynaptic α_2 -adrenoceptor. *J Auton Pharmacol* 1:171-183.

TIMMERMANS, P.B.M.W.M. & VAN ZWIETEN, P.A. 1982. α_2 -adrenoceptors: classification, localization, mechanisms, and targets for drugs. *Journal of Medicinal Chemistry* 25:1389-1401.

VAN MEEL, J.C.A. 1982. The vascular postjunctional adrenoceptor. *Rodopi Amsterdam* (Thesis).

WEITZELL, R., TANAKA, T. & STARKE, K. 1979. Pre- and postsynaptic effects of yohimbine stereoisomers on noradrenergic transmission in the pulmonary artery of the rabbit. *Neunyn-Schmiedeberg's Arch Pharmacol* 308:127-136.

WIKBERG, J.E.S. 1978. Pharmacological classification of adrenergic α -receptors in guinea pig. *Nature Vol* 273, No 5658, pp 164-166.

WIKBERG, J.E.S. 1979. The pharmacological classification of adrenergic α_1 - and α_2 -receptors and their mechanisms of action. *Acta Physiol Scand, Suppl.* 468.

LANGER, S.Z. & SHEPPERSON, N.B. 1981. Subclassification of adrenoceptors into α_1 - and α_2 -categories: relevance to anti-hypertensive therapy. In: New trends in arterial hypertension. Intern Symposium No 17 (ed. M. Worcel et al.), pp. 73-85. Elsevier-North Holland Biomedical Press.

McGRATH, J.C. 1982. Evidence for more than one type of post-junctional adrenoceptor. *Biochem Pharmacol* 31:467-484.

OHMORI, K. & HARI, K. 1979. Restoration of skin cover by free flap transfer. In: Operative Surgery, Fundamental International Techniques, Plastic Surgery. Butterworths & Co, London, 1979.

ROBINS, D.W., 1983. Alpha-adrenergic blockade in prolonged surgery. *Research and Clinical Forums*, 5, no 2, 1983.

RUFFOLO, R.R. Jr. 1983. Alpha-adrenoceptors. In: Neuroreceptors in Health & Disease. Ed. J. Marwaha and W. Anderson. S. Karger AG, Basel, 1983.

RUFFOLO, R.R., WADDELL, J. E & YADEN, E.L. 1982. Heterogeneity of postsynaptic alpha adrenergic receptors in mammalian aortas. *J Pharmacol Exp Ther* 221:309-314.

RUFFOLO, R.R., WADDELL, J.E. & YADEN, E.L. 1981. Postsynaptic alpha-adrenergic receptor subtypes differentiated by yohimbine in tissues from the rat. Existence of α_2 adrenergic receptors in rat aorta. *J Pharmacol Exp Ther* 217:235-240.

SKÄRBY, T.V.C., ANDERSSON, K-E. & EDVINSSON, L. 1983. Pharmacological characterization of postjunctional α -adrenoceptors in isolated feline cerebral and peripheral arteries. *Acta Physiol Scand* 117:63-73.

STARKE, K. 1981. α - Adrenoceptor subclassification. *Rev Physiol Biochem Pharmacol*. Vol 88. Springer Verlag.

Table I

Data on the Schild regressions. NA was used as agonist. The values represent mean and the 95 % confidence interval. Each regression line was determined from 17 - 18 values.

Vessels	Agonist	Slope of regression line	$pA_{2\text{-value}}$
Arteries	Prazosin	0.94 (0.76 - 1.13)	9.86 (9.52 - 10.35)
Veins	Rauwolscine	0.82 (0.63 - 1.01)	9.03 (8.61 - 9.68)

Table II

Effects of α -adrenoceptor agonists on isolated human groin arteries and veins. Values represent mean with 95 % confidence intervals and n denotes number of patients

Agonist	n	Arteries		Veins	
		pEC ₅₀	E _{max} (% of K ⁺)	n	pEC ₅₀ E _{max} (% of K ⁺)
Phenylephrine	6	6.41 (5.90-6.92)	120 (109-130)	6	5.34 (4.88-5.80) 72 (30-114)
Clonidine	6	-	< 5	6	6.24 (5.85-6.63) 27 (0-56)
Oxymetazoline	6	7.29 (6.21-8.37)	65 (6-124)	6	6.76 (5.90-7.62) 86 (59-113)
Noradrenaline	6	6.23 (5.81-6.64)	136 (100-178)	6	6.77 (6.01-7.53) 91 (69-113)

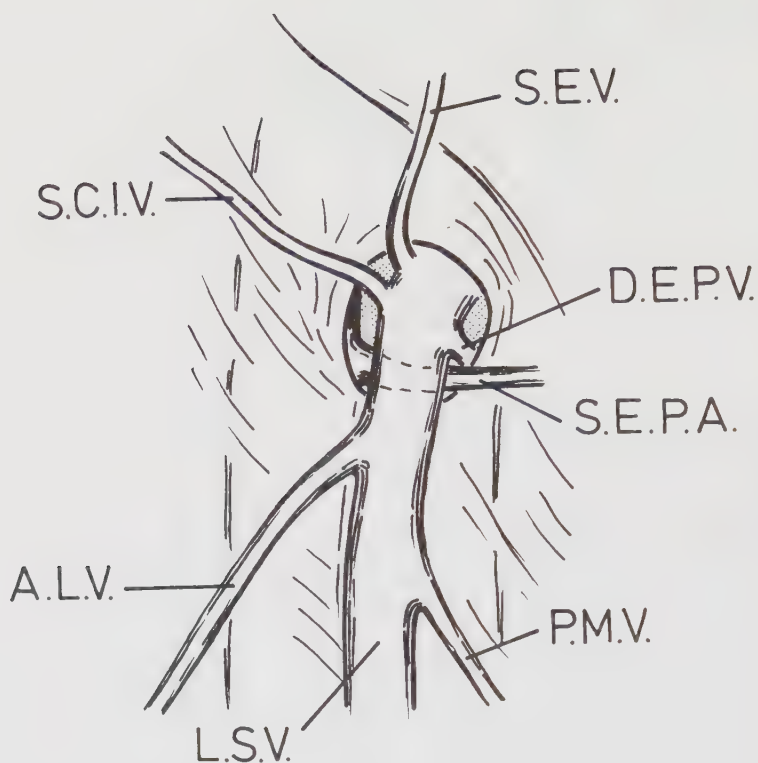


Fig 1

Schematic drawing of "stella venosum" in the right groin, showing the relationships of the superficial external pudendal artery (S.E.P.A.) to the termination of the long saphenous vein (L.S.V.). S.C.I.V.= Superficial circumflex iliac vein. S.E.V.=Superficial epigastric vein. D.E.P.V.= Deep external pudendal vein. P.V.M.= Posteromedial vein of the thigh. A.L.V.= Antero-lateral vein of the thigh. The superficial external pudendal vein is not drawn for the sake of clarity.

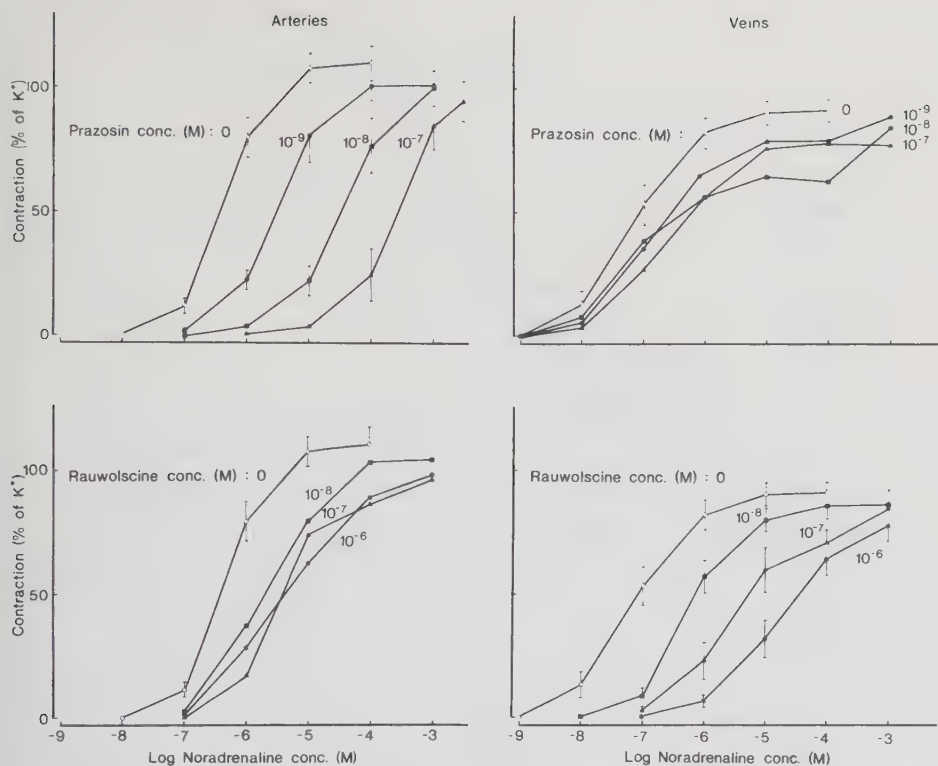


Fig 2

Concentration-response curves for NA in the absence and presence of increasing concentrations of prazosin (upper panel) and rauwolscine (lower panel). Arteries are shown to the left, and veins to the right. The contractile responses are expressed as percentage of the K⁺ (124 mM) -induced contraction. Each point in the curves indicates mean $\pm$ standard error of results obtained from 6 patients.

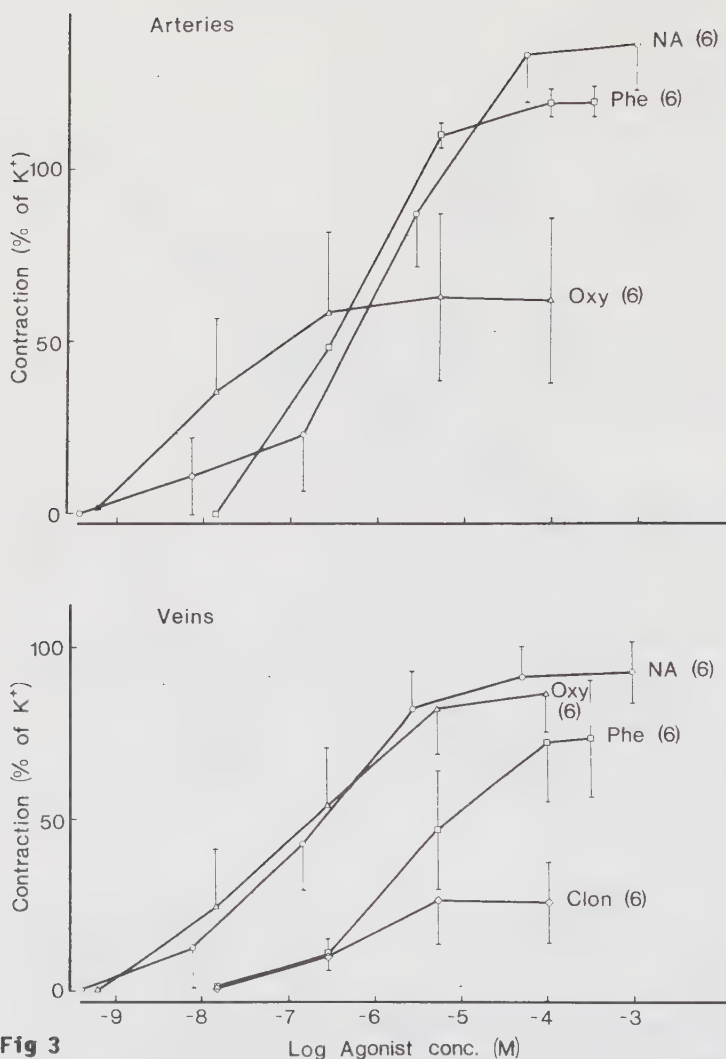


Fig 3

Concentration-response curves for noradrenaline (NA, o), phenylephrine (Phe, □), clonidine (Clon, ◇) and oxymetazoline (oxy, △) in human groin arteries and veins. The effects of all drugs were examined in vessels from six patients. Each patient indicates mean $\pm$ standard error and is expressed as percentage of the K^+ (124 mM)-induced contraction.

THE POSTJUNCTIONAL α -ADRENOCEPTORS OF THE HUMAN SAPHENOUS VEIN

Stig Steen, Trygve Sjöberg, Tor Skärby, Lars Norgren and
Karl-Erik Andersson.

Departments of Surgery and Clinical Pharmacology, University of
Lund, Lund, Sweden.

Running title: α -Adrenoceptors in human saphenous vein.

Please send correspondence to: Stig Steen, M.D.
Department of Surgery
University of Lund
S-221 85 LUND
Sweden

ABSTRACT

The postjunctional α -adrenoceptors in human long saphenous vein were characterized using α -adrenoceptor subtype selective agonists and antagonists. The order of potency for the α -adrenoceptor agonists used was: Clonidine (α_2) > BHT-920 (α_2) > naphazoline (α_2) > guanfacine (α_2) > cirazoline (α_1) > phenylephrine (α_1) > ST 587 (α_1) > BHT-933 (α_2). Clonidine had the same potency as noradrenaline (NA), but was 52 times more potent than phenylephrine (pEC_{50} 7.09 and 5.37, respectively). Phenylephrine and guanfacine had intrinsic activities that did not differ from that of NA, whereas the intrinsic activities of the other agonists were significantly lower. The highly selective α_1 -adrenoceptor antagonist prazosin in concentrations 10^{-9} - 10^{-7} M was unable to cause a significant shift of the NA concentration response (cr) curve to the right. However, the α_2 -adrenoceptor antagonists yohimbine and rauwolscine in concentrations 10^{-8} - 10^{-6} M shifted the NA cr-curve towards higher concentrations. No concentration of any antagonist used significantly attenuated the maximum contraction. The slope of the regression line in the Schild plot differed significantly from unity for rauwolscine but not for yohimbine, and the pA_2 -values were 9.00 and 8.27, respectively. These results suggest that the contraction mediating α -adrenoceptors in the human saphenous vein are mainly of the α_2 -type. However, the low slope of the Schild plot for rauwolscine may indicate the presence of a small population of α_1 -adrenoceptors.

Key words: Human saphenous vein, α -adrenoceptor subtypes, α_1 -adrenoceptors, α_2 -adrenoceptors.

INTRODUCTION

The long saphenous vein is an important vessel in cardiovascular surgery. It is the graft of choice in coronary and femoropopliteal by-passes as well as in peripheral vascular trauma surgery. Furthermore, the vessel is much used, particularly in developing countries, in order to obtain a safe intravenous route. Traumatic dissection of the vein elicits a pronounced contraction of the vessel making the cannulating procedure or the performance of vessel anastomoses difficult. Thus, it is of obvious clinical interest to obtain information on the factors regulating contraction and relaxation in this vessel.

Several investigations on vascular smooth muscle from animals and man have suggested that even if one subtype of α -adrenoceptor can predominate in a particular region, both α_1 - and α_2 -adrenoceptors often occur together and both can mediate contraction (Timmermans & van Zwieten 1981, 1982, Mc Grath 1982, van Meel 1982, Elliott & Reid 1983, Ruffolo 1983). Thus, the isolated dog saphenous vein, studied in vitro, was suggested to be endowed with both α_1 - and α_2 -adrenoceptors postjunctionally (De Mey & Vanhoutte 1981, Shepperson & Langer 1981, Constantine et al 1982). Whether this is valid for the human saphenous vein has not been established.

The present study was therefore performed in order to characterize the postjunctional α -adrenoceptor population in the human long saphenous vein, using α -adrenoceptor subtype selective agonists and antagonists.

MATERIAL AND METHODS

Patients, preparation and mounting

Seventeen patients (11 women and 6 men, aged 31 - 65 years, mean 48 years) undergoing surgery because of primary varicose veins of the long saphenous system were chosen as vessel donors. All of them were healthy and normotensive without drug treatment of any kind. Thirteen operations were performed under epidural anesthesia, whereas four were carried out under thiopental-halothane anesthesia. The long saphenous vein was dissected free at the medial malleolus, care being taken to avoid stretching or other type of injury. Only patients with normal ankle saphenous veins (macroscopically) were chosen as donors. A vein segment of 1 - 2 cm length was excised and immediately placed in chilled Krebs solution. Under an operation microscope the vessel was dissected free from fat and cut into ring segments of 1 - 2 mm length. Some of these segments were immediately mounted in temperature-controlled organ baths, each with a volume of 5 ml. The rest were kept in Krebs solution (refrigerator) for later use (within 24 h). The Krebs solution had the following composition (in mM): NaCl 119, NaHCO₃ 15, KCl 4.6, CaCl₂ 1.5, NaH₂PO₄ 1.2, MgCl₂ 1.2 and glucose 11. Cocaine, 10⁻⁷ M (for blockade of neuronal uptake), and propranolol, 10⁻⁷ M (to block β -adrenoceptors), were also added. In the baths, the buffer solution was continuously bubbled with a mixture of 95 % O₂ and 5 % CO₂ giving a pH of approximately 7.4. The temperature was maintained at 37° C.

The vessel segments were suspended between two L-shaped metal holders (0.2 mm in diameter), one of which was attached to a Grass FT 03 transducer connected to a Grass polygraph for continuous recording of isometric tension. The position of the other holder could be changed by means of a movable unit allowing fine adjustments of vessel tension by varying the distance between the metal prongs (for further experimental details, see Högestätt et al 1983).

A passive tension of 8 mN was applied to the vessel segments resulting in a relaxation which was compensated for by stretching them back to a tension of 8 mN. In separate experiments, where the vessels were contracted with a K^+ -rich Krebs solution at different basal tensions, it was ascertained that an optimum contractile response was obtained at this basal tension.

When a steady level of 8 mN had been obtained (after 1-2 h) the vessels were contracted by exposure to a K^+ -rich Krebs solution containing (in mM): KCl 124, $NaHCO_3$ 15, $CaCl_2$ 1.5, NaH_2PO_4 1.2, $MgCl_2$ 1.2, glucose 11.

Investigations

The vessels were repeatedly exposed to a K^+ -rich Krebs solution until the variation between two subsequent contractions was less than 10 %. This was generally obtained after 3 or 4 exposures (10 min each 1/2 h intervals). After further 45 min equilibration in Krebs solution agonists were added cumulatively and concentration-response (cr) curves were constructed on basis of the data obtained.

The experiments with antagonists were performed by studying 4 or 5 vein segments simultaneously. After NA cr-curves had been obtained in all vessels, one vein segment was exposed to prazosin, another to rauwolscine, and a third to yohimbine, whereas 1 or 2 segments served as controls. The antagonists were in contact with the tissue for 15-20 minutes prior to the cumulative addition of NA. After NA cr-curves had been obtained, all segments were washed in Krebs solution for a minimum of 45 minutes. The same procedure was thereafter repeated twice, but now with ten respectively hundred times higher antagonist concentrations. In the control segments the reproducibility of the NA cr-curves was examined in parallel.

The EC_{50} -value (i.e. the agonist concentration at which 50% of maximum effect occurs) for NA in the control segments served as reference to which the EC_{50} -values of the other segments in the same experiment were compared when calculating the concentration ratios for the pA_2 -determinations (see below).

The K^+ -induced contraction was used as an internal standard in each segment, and each response was expressed as a percentage of this contraction. The amplitude of these contractions was 22.0 ± 4.1 mN (mean $\pm$ SEM $n=17$). In separate experiments it was observed that pretreatment with phentolamine (10^{-6} M) reduced the K^+ -induced contraction by less than 10%. It was also shown that an increase in cocaine or propranolol concentrations beyond those used in this investigation did not increase the potency or maximum response to NA ($n=6$).

Analysis of data

All cr-curves were plotted graphically and E_{max} , i.e. the maximum contraction obtained with an agonist, and pEC_{50} , i.e. the negative logarithm of the EC_{50} -value, were calculated. With those antagonist concentrations in presence of which the NA cr-curve did not reach a well defined plateau, the pEC_{50} -values were calculated assuming E_{max} to be unchanged as compared to the previous NA cr-curve where a plateau was reached.

The pA_2 -value, i.e. the negative logarithm of the antagonist concentration that displaced the agonist cr-curves towards higher concentrations with a factor of 2, was determined according to the method of Arunlakshana and Schild (1959) (Schild plot).

The concentration ratio (CR) was calculated as the ratio of EC_{50} -values in presence and absence of antagonist.

Statistics

Paired or unpaired Student's t -test were used to determine statistical significance. The regressions of the Schild plots,

based on linear least square fit, were performed on a calculator (Hewlett Packard 41 CV) using a program kindly supplied by Dr. Claus Rerup, Department of Pharmacology, University of Lund.

The results from the Schild plots are given as mean with 95 % confidence intervals. The other results are given as mean $\pm$ SEM with n referring to the number of vessels, each of which was excised from a different patient. (If more than one value was obtained from the same patient the mean of these were included in the results).

Drugs

BHT-920 (6-alkyl-2-amino-5,6,7,8-tetrahydro-4H-thiazolo- 4,5-d -azepine dihydrochloride), BHT-933 (azepepexole; 2-amino-6-ethyl-5,6,7,8,-tetrahydro-4H-oxazolo- 4,5-d -azepine dihydrochloride) (Thomae), cirazoline (LD 3098) (Synthelabo), clonidine hydrochloride, cocaine hydrochloride (Merck), guanfacine hydrochloride (Sandoz), naphazoline chloride (Ciba-Geigy), ($\pm$)-noradrenaline hydrochloride, (-)-phenylephrine hydrochloride, yohimbine hydrochloride (Sigma), prazosin hydrochloride (Pfizer), ($\pm$)-propranolol hydrochloride (ICI), rauwolscine hydrochloride (Roth), ST-587 (2-(2-chloro-5-trifluoromethylphenylimino) imidazoline, nitrat) (Boehringer Ingelheim).

The drugs were dissolved in 0.9% NaCl containing 1.0 mM ascorbic acid except prazosin, which in the concentration 1 mM was dissolved in 10% lactic acid or 1 mM HCl and 1 % methanol. Stock solutions of the drugs were prepared and stored at -70 °C until use. Only fresh dilutions (in 0.9% NaCl and 1.0 mM ascorbic acid) were used. The concentrations are given as final molar concentrations in the organ baths.

RESULTS

Effects of α -adrenoceptor agonists

The cr-curves for the α -adrenoceptor agonists used are shown in Fig 1 and the pEC₅₀- and E_{max}-values are given in Table I. Phenylephrine and guanfacine had E_{max} values that did not significantly differ from that of NA, whereas those of all other agonists were significantly lower. The relative order of potency was: Clonidine > BHT-920 > naphazoline > guanfacine > cirazoline > phenylephrine > ST-587 > BHT 933.

Effects of α -adrenoceptor antagonists

As can be seen in Fig 2 the NA cr-curves were well reproducible during several subsequent cumulative additions of NA.

No concentration of any antagonist used, significantly attenuated E_{max}. Although the NA cr-curve did not reach a well defined plateau with 10⁻⁶ M yohimbine or rauwolscine present in the bath, the contraction elicited by 10⁻³ M NA in presence of this concentration of antagonists did not differ significantly from the E_{max} in absence of antagonist. Prazosin in the concentration 10⁻⁹ M appeared to shift the NA cr-curve to the right. However, the shift was not significant. The concentrations 10⁻⁸M and 10⁻⁷ M caused little or no further shift (Fig 3). It was therefore not considered relevant to calculate a Schild plot based on these results.

Rauwolscine as well as yohimbine caused concentration dependent shifts of the NA cr-curves towards higher concentrations (Fig 3). Graphical illustration of the interaction between NA and rauwolscine/yohimbine in Schild plots resulted in a slope of 0.68 for rauwolscine and 0.86 for yohimbine. The corresponding pA₂ - values were 9.00 and 8.27 respectively (Fig 4, table II). The slope for rauwolscine but not yohimbine differed significantly (p < 0.02) from unity.

DISCUSSION

The agonists phenylephrine, cirazoline, and ST-587 are considered to have a preference for α_1 -adrenoceptors whereas clonidine, BHT-920, naphazoline, guanfacine and BHT-933 are regarded to preferentially stimulate α_2 -adrenoceptors (Kobinger & Pichler 1980, 1981a, Timmermans & van Zwieten 1980, 1982, Langer & Shepperson 1982, Ruffolo 1983).

The potency ratios of clonidine/phenylephrine and NA/phenylephrine in the present study agree well with those proposed to be representative for α_2 -adrenoceptors (Starke 1981). Furthermore, the order of potency of rauwolscine, yohimbine and prazosin is the same as that suggested to represent an interaction with α_2 -adrenoceptors (e.g. Langer 1980, Langer & Shepperson 1981, Starke 1981). Also the pA_2 -value of yohimbine (8.27) correlates well with the pA_2 -values obtained in several studies where yohimbine was considered to interact with α_2 -adrenoceptors. These values ranged between 7.3 and 8.7 (De Mey & Vanhoutte 1981, Glusa & Markwardt 1983, Weiss et al 1983, Drew 1978, Fuder et al 1983, Lavin et al 1981, Tharp et al 1981, Brodde et al 1982, Motulsky & Insel 1982, Barnes et al 1983, Sakakibara et al 1982). Taken together, these results suggest that the α -adrenoceptor mediating contraction in the human saphenous vein is mainly of the α_2 -subtype. The finding that the α_2 -adrenoceptor agonists BHT-920, naphazoline and guanfacine were more potent than the α_1 -adrenoceptor agonists cirazoline and phenylephrine further supports this conclusion.

Although BHT-933 has been found to preferentially stimulate α_2 -adrenoceptors the potency of this agonist in activating α_2 -adrenoceptors has been reported to be low (Kobinger & Pichler 1981b, van Meel 1982). In the present study, BHT-933 was found to have the lowest potency of the agonists examined. The value of this drug as a tool for investigating the α -adrenoceptors in the human saphenous vein may therefore be questioned.

Yohimbine and the yohimbine stereoisomer rauwolscine are recognized as two of the most selective α_2 -adrenoceptor antagonists presently available (Ruffolo 1983). In the present study both drugs caused concentration-dependent shifts of the NA cr-curves without significantly affecting $E_{\max}$, suggesting a reversible competitive interaction.

The slope of the Schild plot for yohimbine did not differ from unity, implying an interaction with one type of receptor (Arunlakshana & Schild 1959, Kenakin 1982). However, the slope for rauwolscine differed significantly from 1. A slope factor less than unity would be expected if the NA uptake mechanisms were not sufficiently blocked (Furchgott 1972, Kenakin 1982). The use of high cocaine concentrations (10^{-5} M) did, however, not shift the NA cr-curve leftwards. This finding agrees well with the results of Janssens & Verhaeghe (1983) in a human saphenous vein preparation. These authors also found that hydrocortisone 3×10^{-7} - 3×10^{-5} M had no effect on the NA cr-curves (Janssens & Verhaeghe 1983). These results indicate that the low slope factor of the Schild plot for rauwolscine found in the present investigation was not due to an inadequate blockade of amine uptake mechanisms. Kenakin (1982) suggested that the slope of a Schild plot may be lower than unity if an agonist activates two types of receptor producing the same type of response and the antagonist blocks only one of these. Thus, an explanation for the low slope factor may be that, besides the main population of α_2 -adrenoceptors, there is a small population of α_1 -adrenoceptors. The tendency of prazosin to cause a shift of the NA cr-curve may also be due to this α_1 -adrenoceptor population. Indeed, this interpretation correlates well with recent in vivo results obtained on the human saphenous vein (Steen et al 1984) showing that low concentrations ($< 10^{-8}$ M) of prazosin significantly attenuated vasoconstriction elicited by NA. It may be speculated that the slope of the Schild plot for yohimbine did not differ from unity because this antagonist is less selective than

rauwolscine (McGrath 1982) and thus less able to distinguish between the two types of receptor.

In conclusion, the contraction-mediating α -adrenoceptors of the human long saphenous vein appear to be mainly of the α_2 -type, although a small population of α_1 -adrenoceptors cannot be excluded.

REFERENCES

1. Arunlakshana O, Schild HO (1959) Some quantitative uses of drug antagonists. *Brit J Pharmacol* 14:48-59
2. Barnes PJ, Skoogh B-E, Nadel JA, Roberts JM (1983) Postsynaptic α_2 -adrenoceptors predominate over α_1 -adrenoceptors in canine tracheal smooth muscle and mediate neuronal and hormonal alpha-adrenergic contraction. *Mol Pharmacol* 23:550-575
3. Brodde O-E, Hardung A, Ebel H, Bock KD (1982) GTP regulates binding of agonists to α_2 -adrenergic receptors in human platelets. *Arch Int Pharmacodyn* 258:193-207
4. Constantine JW, Lebel W, Archer R (1982) Functional postsynaptic α_2 - but not α_1 -adrenoceptors in dog saphenous vein exposed to phenoxybenzamine. *Eur J Pharmacol* 85:325-329
5. De Mey J, Vanhoutte PM (1981) Uneven distribution of postjunctional α_1 - and α_2 -like adrenoceptors in canine arterial and venous smooth muscle. *Circ Res* 48:875-884
6. Drew GM (1978) Pharmacological characterization of the presynaptic α -adrenoceptors regulating cholinergic activity in the guinea-pig ileum. *Br J Pharmacol* 64:293-300
7. Elliott HL, Reid JL (1983) Evidence for postjunctional vascular α_2 -adrenoceptors in peripheral vascular regulation in man *Clin Sci.* 65:237-241

8. Fuder H, Muschoff E, Spemann R (1983) The determination of presynaptic pA_2 values of yohimbine and . phentolamine on the perfused rat heart under conditions of negligible autoinhibition. *Br J Pharmacol* 79:109-119
9. Furchgott, R.F. The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory.. In *Handbook of Experimental Pharmacology* (Eds. H. Blaschko & E. Muscoll), pp. 283-355, Springer Verlag, New York, 1972.
10. Glusa E, Markwardt F (1983) Characterization of postjunctional α -adrenoceptors in isolated human femoral veins and arteries. *Naunyn-Schmiedeberg's Arch Pharmacol* 323:101-105
11. Högestätt ED, Andersson K-E, Edvinsson L (1983) Mechanical properties of rat cerebral arteries as studied by a sensitive device for recording of mechanical activity in isolated small blood vessels. *Acta Physiol Scand* 117:49-61
12. Janssens W, Verhaeghe R (1983) Modulation of the concentration of noradrenaline at the neuro-effector junction in human saphenous vein. *Br J Pharmacol* 79:577-585
13. Kenakin TP (1982) The Schild regression in the process of receptor classification. *Can J Physiol Pharmacol* 60:249-265

14. Kobinger W, Pichler L (1980) Relation between central sympathoinhibitory and peripheral pre- and postsynaptic α -adrenoceptors as evaluated by different clonidine-like substances in rats. *Naunyn-Schmiedeberg's Arch Pharmacol* 315:21-27
15. Kobinger W, Pichler L (1981a) α_1 - and α_2 -adrenoceptor subtypes: selectivity of various agonists and relative distribution of receptors as determined in rats. *Eur J Pharmacol* 73:313-321
16. Kobinger W, Pichler L (1981b) α_2 -Adrenoceptor blockade by α_2 -adrenoceptor agonists in the isolated perfused hindquarter of rats. *Eur J Pharmacol* 72:113-115
17. Langer SZ (1980) Presynaptic receptors and modulation of neurotransmission: pharmacological implications and therapeutic relevance. *Trends Neurosci* May:110-112
18. Langer SZ & Shepperson NB (1981) Subclassification of α -adrenoceptors into α_1 - and α_2 -categories: relevance to antihypertensive therapy. In: *New trends in arterial hypertension. Inserm Symposium No 17* (ed M Worcel et al) pp 73-85. Elsevier/North Holland Biomedical Medical Press.
19. Langer SZ, Shepperson NB (1982) Recent developments in vascular smooth muscle pharmacology: The post-synaptic α -adrenoceptors. *Trends Pharmacol Sci* 3:440-444
20. Lavin TN, Hoffman BB, Lefkowitz RJ (1981) Determination of subtype selectivity of alpha-adrenergic antagonists. Comparison of selective and nonselective radioligands. *Mol Pharmacol* 20:28-34

21. McGrath JC (1982) Evidence for more than one type of postjunctional α -adrenoceptors. *Biochem Pharmacol* 31:467-484
22. Motulsky HJ, Insel PA (1982) ^3H dihydroergocryptine binding to alpha-adrenergic receptors of human platelets. A reassessment using the selective radioligands ^3H prazosin, ^3H yohimbine, and ^3H rauwolscine. *Biochem Pharmacol* 31:2591-2597
23. Ruffolo RR, Jr (1982) Review. Important concepts of receptor theory. *J Auton Pharmacol* 2:277-295
24. Ruffolo, RR, Jr. Alpha-adrenoceptors. In *Neuroreceptors in health & disease* (Eds. J. Marwaha and W. Anderson), pp. , S Karger AG, Basel, 1983.
25. Sakakibara Y, Fujiwara M, Muramatsu I (1982) Pharmacological characterization of the alpha-adrenoceptors of the dog basilar artery. *Naunyn Schmiedeberg's Arch Pharmacol* 319:1-7
26. Shepperson NB, Langer SZ (1981) The effects of the 2-amino-tetrahydronaphthalene derivative M7, a selective α_2 -adrenoceptor agonist in vitro. *Naunyn-Schmiedeberg's Arch Pharmacol* 318:10-13
27. Starke K (1981) α -Adrenoceptor subclassification. *Rev Physiol Biochem Pharmacol* 88
28. Steen S, Castenfors J, Sjöberg T, Skärby T, Andersson K-E & Norgren L (1984) Effects of α -adrenoceptor subtype selective antagonists on the human saphenous vein in vivo. In manuscript.

29. Tharp MD, Hoffman BB, Lefkowitz RJ (1981) α -Adrenergic receptors in human adipocyte membranes: direct determination by ^3H yohimbine binding. *J Clin Endocrinol Metabol* 52:709-714
30. Timmermans PBMWM, van Zwieten PA (1980) The postsynaptic α_1 and α_2 adrenoceptors in the circulatory system of the pithed rat. Selective stimulation of the α_2 subtype by BHT-933. *Eur J Pharmacol* 63:199-202
31. Timmermans PBMWM, van Zwieten PA (1981) The postsynaptic α_2 -adrenoceptor. *J Auton Pharmacol* 1:171-183
32. Timmermans PBMWM, van Zwieten PA (1982) α_2 -Adrenoceptors: Classification, localization, mechanisms and targets for drugs. *J Med Chem* 25:1389-1401
33. van Meel JCA (1982) The vascular postjunctional α_2 -adrenoceptor. *Rodopi Amsterdam (Thesis)*.
34. Weiss RJ, Webb RC, Smith CB (1983) α_2 -adrenoceptors on arterial smooth muscle: Selective labeling by ^3H clonidine. *J Pharmacol Exp Ther* 225:599-605

Table I.

Effects of α -adrenoceptor agonists on isolated human saphenous vein.

Values represent mean and standard error of mean (SEM) and n number of different preparations.

α -receptor agonists	α -receptor preferences	n	pEC_{50}	E_{max} (% of K^+)
Clonidine	$\alpha_2 > \alpha_1$	6	7.09 ± 0.11	57 ± 9
Noradrenaline	$\alpha_2 = \alpha_1$	14	6.99 ± 0.09	109 ± 6
BHT-920	$\alpha_2 > \alpha_1$	6	6.88 ± 0.21	69 ± 8
Naphazoline	$\alpha_2 > \alpha_1$	6	6.57 ± 0.15	64 ± 10
Guanfacine	$\alpha_2 > \alpha_1$	6	6.12 ± 0.17	91 ± 7
Cirazoline	$\alpha_1 > \alpha_2$	6	6.06 ± 0.19	57 ± 8
Phenylephrine	$\alpha_1 > \alpha_2$	6	5.37 ± 0.31	97 ± 11
ST-587	$\alpha_1 > \alpha_2$	6	5.32 ± 0.23	19 ± 4
BHT-933	$\alpha_2 > \alpha_1$	6	5.18 ± 0.09	49 ± 11

Table II.

Data on the Schild regressions. Noradrenaline was used as agonist.

The values represent mean and the 95% confidence interval, n denotes number of patients and z represents the number of values on which the regression line is based.

n	Antagonist	Slope of regression line	z	pA_2 -value
7	Rauwolscine	0.68 (0.44-0.92)	20	9.00 (8.45-10.12)
6	Yohimbine	0.86 (0.58-1.14)	18	8.27 (7.89- 8.96)

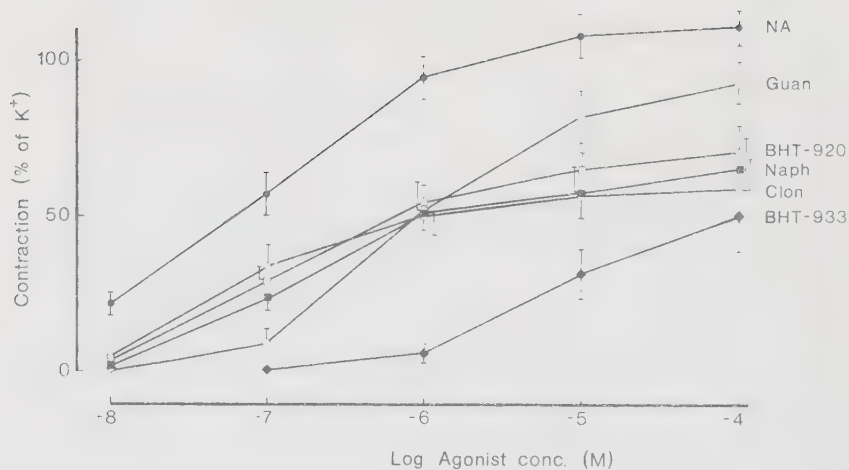


Fig 1a. Concentration-response curves for noradrenaline (NA, ●) and the α_2 -adrenoceptor agonists guanfacine (Guan, ◇) BHT-920 (□), naphazoline (Naph, ■), clonidine (Clon, ○), and BHT-933 (◆) in human saphenous vein. Each point indicates mean $\pm$ SEM and is expressed as percentage of the K^+ (124 mM)-induced contraction. $n = 6$ except for NA in which $n = 14$.

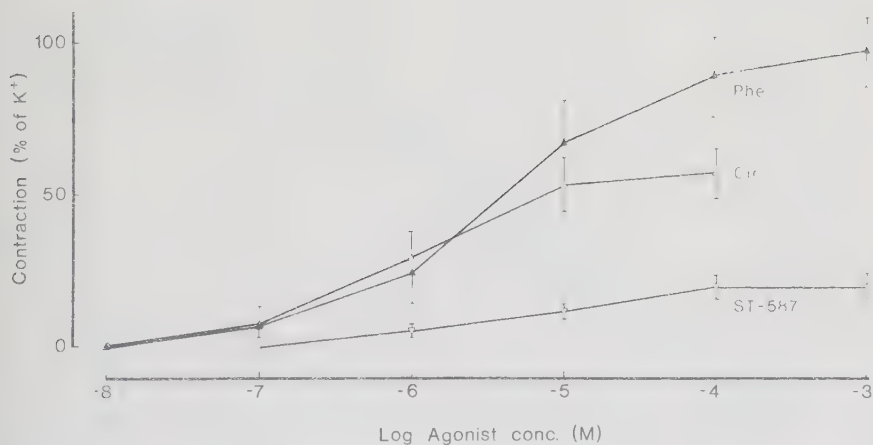


Fig 1b. Concentration-response curves for the α_1 -adrenoceptor agonists phenylephrine (Phe $\blacktriangle$), cirazoline (Cir $\triangle$) and ST-587 (∇). Each point represents mean $\pm$ SEM of 6 experiments and is expressed as percentage of the K⁺ (124 mM)-induced contraction.

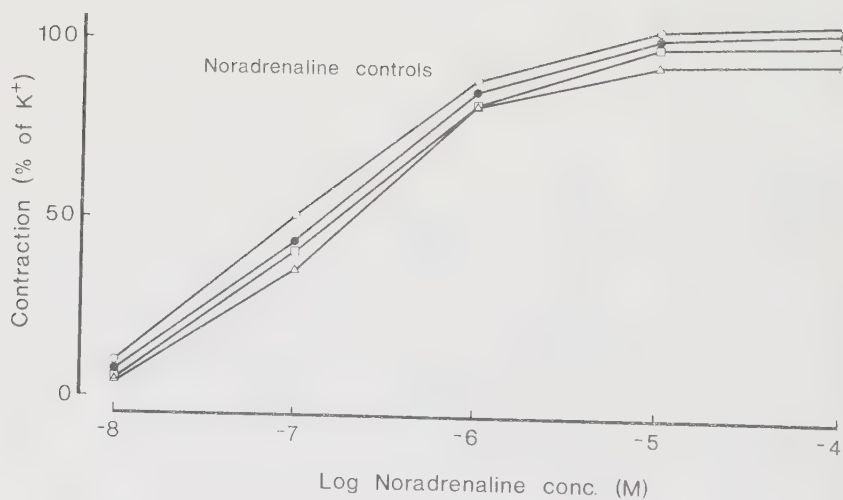


Fig 2. Concentration-response curves for noradrenaline controls from human saphenous vein, obtained in the following order: $\circ$ $\bullet$ $\square$ $\triangle$ Each point indicates mean ($n=9$) and is expressed as percentage of K^+ (124 mM)-induced contraction.

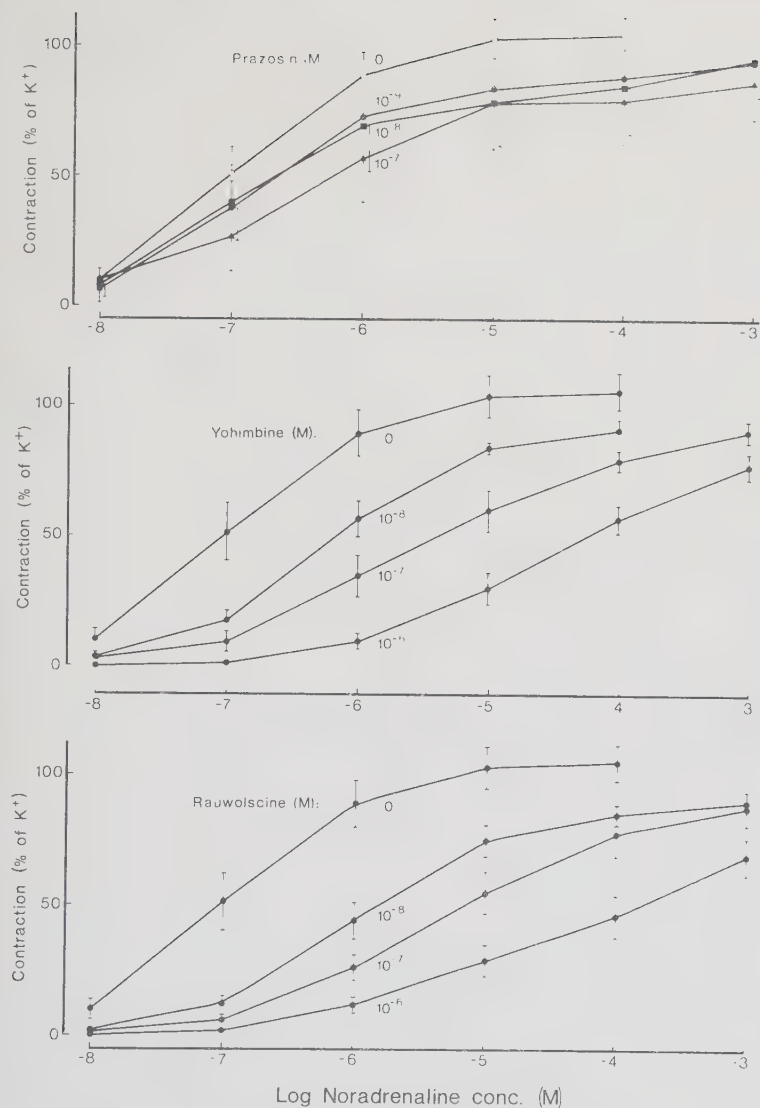


Fig 3. Concentration-response curves for NA in the absence and presence of increasing concentrations of prazosin (upper panel, $n=8$), yohimbine (middle panel, $n=6$) and rauwolscine (lower panel, $n=7$). The

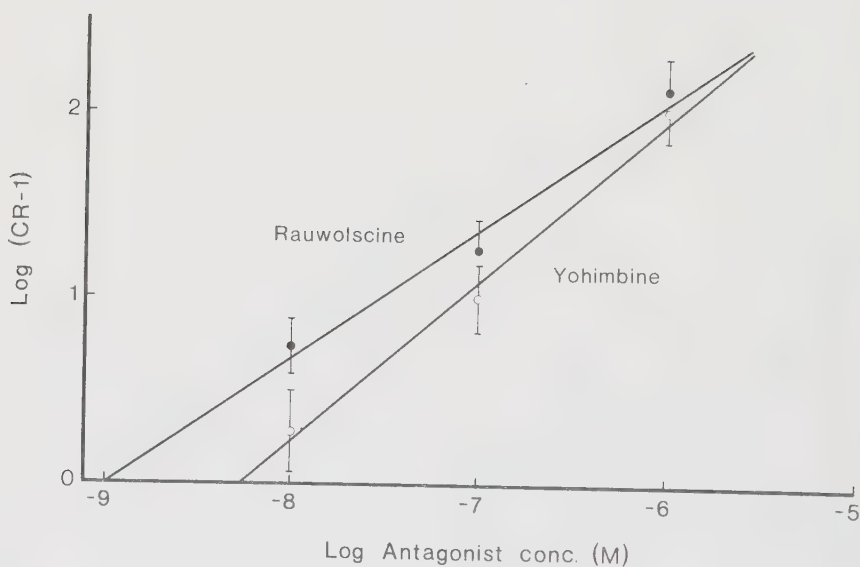


Fig 4. Effects of rauwolscine (●) and yohimbine (○) on NA-responses of human saphenous vein as illustrated by Schild plots. Each regression is calculated from vessels of 6 (yohimbine) or 7 (rauwolscine) patients and each point denotes mean $\pm$ standard error.

EFFECTS OF α -ADRENOCEPTOR SUBTYPE SELECTIVE ANTAGONISTS
ON THE HUMAN SAPHENOUS VEIN IN VIVO

Stig Steen, Jan Castenfors, Trygve Sjöberg, Tor Skärby,
Karl-Erik Andersson and Lars Norgren

Departments of Surgery, Clinical Physiology and Clinical
Pharmacology, University of Lund, Sweden.

Please send correspondence to: Stig Steen, M.D.

Department of Surgery
University of Lund
S-221 85 LUND
Sweden

ABSTRACT

The effects of the α -adrenoceptor subtype selective antagonists prazosin (α_1) and yohimbine (α_2) were investigated in the saphenous vein of 6 healthy male subjects.

The drugs were infused locally into the congested (40 mm Hg) long saphenous vein precontracted by simultaneous local infusion of noradrenaline (NA). Prazosin 10^{-9} M (concentration in infusion solution; infusion rate 0.3 ml/min) caused no blockade of the NA-induced venoconstriction, but at a concentration of 10^{-8} M the drug consistently caused a significant reduction; in two subjects, no response to NA could be elicited in the presence of 10^{-8} M prazosin. Prazosin 10^{-7} M caused no further blockade compared to that produced by 10^{-8} M in three of the subjects, whereas in one, prazosin 10^{-9} , 10^{-8} and 10^{-7} M caused a dose-dependent inhibition. Yohimbine, 10^{-9} , 10^{-8} and 10^{-7} M (concentrations in infusion solutions) caused a dose-dependent blockade of the NA-induced venoconstriction in all subjects.

The study suggests that the human saphenous vein is endowed with functionally important populations of both α_2 - and α_1 -adrenoceptors.

Key words: Human saphenous vein - α -adrenoceptor subtypes - α_1 -adrenoceptors, α_2 -adrenoceptors

INTRODUCTION

De Mey and Vanhoutte (1980; 1981) studying isolated canine arteries and veins, found predominantly α_1 -adrenoceptors (α_1 -receptors) postjunctionally in femoral and splenic arteries, whereas in femoral and saphenous veins functionally important postjunctional α -receptors of both α_1 - and α_2 -subtype were demonstrated. These differences between arteries and veins in the distribution of α -receptor subtypes did not seem to be related to the density of adrenergic innervation since they were observed independently of the magnitude of the contractile response to electrical stimulation (De Mey & Vanhoutte 1981).

Shoji et al (1983) investigated isolated venous strips from fifteen different regions in the dog. They found that the potency of the α_2 -receptor agonist clonidine was higher than that for the α_1 -receptor agonist phenylephrine in the saphenous, cephalic, femoral and external jugular veins; in longitudinal cut strips of mesenteric veins the potency of the two agonists was comparable whereas phenylephrine was more potent than clonidine in longitudinally cut strips of the portal vein and in the infra-hepatic suprarenal vena cava. Unlike the longitudinally cut strips, the helically cut strips of the portal and mesenteric veins did not respond to clonidine (Shoji 1983). These results suggest that isolated canine veins have functionally important postjunctional α_2 -receptors. Furthermore, there seem to be regional variations with respect to postjunctional α -receptor subtypes in the venous system.

In man, in vitro studies of metacarpal (Stevens & Moulds 1981, see Flavahan & Mc Grath 1984), femoral (Glusa & Markwardt 1983), omental, groin and saphenous veins (Steen et al 1984 a,b,c) suggest that these vessels are endowed with functionally important populations of postjunctional α_2 -receptors. However, to what extent this conclusion is valid also in vivo has hitherto not been established.

The present study was performed in order to obtain information concerning the influence of α -receptor subtype selective antagonists on the noradrenaline induced contraction in the human saphenous vein in vivo and to compare the results obtained with previous in vitro data.

MATERIAL AND METHODS

Six healthy male subjects aged 25-35 years (mean 30 years) participated in the study, which was approved by the Ethics Committee of the University of Lund. All subjects gave written informed consent.

Changes in diameter of the long saphenous vein at the medial malleolus were measured using a modification of the method described by Collier et al (1972). Briefly, the vein was illuminated from one side with 940 nm infrared light through a 0.2 mm vertical slit. On the other side of the vein variations in light intensity induced by changes in diameter of the vein, were measured with a matched phototransistor (Fig 1) (for further methodological details, see Castenfors et al 1984).

The subjects were placed in supine position in a warm room (28-30°C) to minimize endogenous sympathetic tone. The legs were elevated just above the heart level to achieve "collapsed" saphenous veins (zero level). A sphygmomanometer cuff was placed just below the knee and an occlusion pressure of 40 mmHg was applied, inducing a stable expansion of the saphenous vein. Through an intravenous needle (Butterfly-21, internal diameter 0.6 mm), the point of which was placed 3-4 mm distal to the measuring device, infusion of 0.9 % NaCl alone or with addition of different drugs was given with a constant infusion rate of 0.3 ml/min.

Infusion of noradrenaline (NA) in a concentration of 1.6×10^{-7} M (rate: 10 ng/min) induced a mean decrease in vein size of 62 % (range 35-86 %) compared to NaCl in the different experimental runs. After the response to NA had been tested, the α_1 -receptor blocker prazosin (P) or the α_2 -receptor blocker yohimbine (Y) was added to NaCl which was infused for 15 min resulting in a loss of the NA induced contraction (i.e. return to the standardized 40 mmHg - expansion level). Thereafter infusion of NA in presence of 10^{-9} M P or Y was given until a stable level of venocontraction was reached (in about 10 min). This

procedure (NaCl + P or Y followed by NA + P or Y) was repeated twice, but now with 10^{-8} M followed by 10^{-7} M P or Y.

In two subjects (no 5 and 6, Fig 2) 10^{-9} M P and Y was not given. In subject 1-3 P and Y were given on different days at exactly the same location of the long saphenous vein at the medial malleolus, through the same puncture site in the skin. In subject 4 - 6 the needles were placed symmetrically in the long saphenous vein at the medial malleolus on each side, and the experiment was run in parallel with P in one leg and Y in the other.

The effects of P and Y were expressed in percent of the venoconstriction induced by NA alone which was defined as 100 %. In subject 1 repeated doses of NA alone (10 ng/min) were given during 2 1/2 h and no tachyphylaxis was observed. In subject 1 and 4 the effect of NA alone was tested at the end of the experiment after an infusion of pure NaCl had been given in half an hour to wash out P and Y; no tachyphylaxis was seen. No effect of NaCl with or without P or Y was seen on the congested vein.

Drugs

(±)-Noradrenaline HCl, yohimbine HCl (Sigma), and prazosin HCl (Pfizer) were dissolved in 0.9 % NaCl (containing 1 mM ascorbic acid), water, and 1 mM HCl, respectively. Because of the difficulty to dissolve prazosin, a few drops of ethanol were first added to the drug substance. Further dilutions were made with 0.9 % NaCl. Stock solutions of the substances were stored at -70° C until used. All solutions were sterile filtered before infusion. The concentrations reported refer to the concentrations in the syringe.

RESULTS

Fig 2 shows the local effects of intravenous infusion of prazosin and yohimbine on the congested (40 mmHg) saphenous vein, which was precontracted by simultaneous local NA-infusion at a rate of 10 ng/min. Prazosin 10^{-8} M (concentration in the infused solution) caused a significant inhibition of the NA-contraction in the veins of all subjects. In subjects 3 and 4 no NA-contraction was elicited in presence of 10^{-8} M prazosin. Prazosin 10^{-7} M caused no further blockade of the NA-contraction in subjects 2,5 and 6.

Yohimbine (concentrations 10^{-9} , 10^{-8} and 10^{-7} M in the infused solutions) caused a dose-dependent blockade of the NA-contraction and appeared to be less potent than prazosin in blocking the NA-contraction except in subject 1 (Fig 2).

DISCUSSION

In a previous study, we have characterized the postjunctional α -receptors in the human long saphenous vein in vitro (Steen et al 1984c). It was shown that yohimbine caused a concentration dependent parallel rightward shift of the NA concentration-response curve and a Schild plot gave a pA_2 -value of 8.27; prazosin (10^{-9} , 10^{-8} and 10^{-7} M) caused no significant shift of the NA concentration-response curve. This together with the relative potencies of several α -receptor subtype selective agonists, made us conclude that the postjunctional α -receptors in the human long saphenous vein at the medial malleolus were predominantly of α_2 -type although a small population of α_1 -receptors could not be excluded.

In the present in vivo study the human long saphenous vein was examined at the same location as in the in vitro study, i.e. at the medial malleolus. This was believed to be of importance since Holmberg et al (1983) have shown that NA is more potent in the distal than in the proximal part of the human long saphenous vein. This difference in potency might be due to differences in α -receptor content and subtype distribution. Larsson et al (1984) found that NA was more potent in the distal than in the proximal part of the rabbit urethra. Radioligand binding studies showed that the content of α -receptors gradually increased distally in this tissue and that the increase was mainly due to a selective increase in the number of α_2 -receptors.

At a concentration of 10^{-8} M in the infused solution and at an infusion rate of 0.3 ml/min, prazosin caused a significant blockade of the NA-induced venoconstriction (Fig 2). Considering that the infused amounts of the drug were further diluted by the blood before reaching the α -receptors in the vein wall, it seems as if prazosin was more potent in vivo than in vitro. Prazosin is recognized as a highly selective α_1 -receptor antagonist with a selectivity for α_1 -receptors over α_2 -receptors in the order of 100 - 18 500 (Andersson et al 1984, Timmermans & van Zwieten

1981, Ruffolo 1983). The significant effects of infusion of low doses as in the present study, suggest the presence of a small (but functionally important) population of α_1 -receptors in the human saphenous vein. Supporting this view, a tenfold higher concentration in the infused solution (constant infusion rate) produced no further effect. In vitro prazosin 10^{-7} M did not significantly displace the NA-concentration-response curve towards higher concentrations.

Dashwood (1982) studied the binding of ^3H -prazosin to vascular tissue of the rat; dense prazosin binding sites were found in all arterial tissues examined except mesenteric arteries, whereas ^3H -prazosin binding to veins was often absent. This corresponds well with functional in vitro studies, i.e. vascular postjunctional α_1 -receptors dominate in arteries, but in veins often α_2 -receptors are in predominance. Although the population of postjunctional α_1 -receptors in human saphenous vein is small as judged from the in vitro results, it may be of real importance in vivo since prazosin obviously was more potent in vivo than in vitro. It might be speculated that the reason to this discrepancy was differences in the experimental conditions, e.g. the in vitro contractions were recorded isometrically whereas in vivo isobaric contractions were measured (40 mmHg). Gillard et al (1982) investigated the short- and medium-term hemodynamic effects of prazosin in 10 patients with chronic stable cardiac failure. They found that in short term, prazosin had a mixed vasodilator effect on both arteries and veins. In the medium term (i.e. after 7 days treatment) the arterial vasodilator action appeared to be significantly smaller, but remained detectable with doses greater than 8 mg prazosin/day, whereas the venous vasodilator effect persisted unchanged. Even if the disease and the previous treatment may have changed the responses to prazosin in the patients, this study suggests that prazosin does act on human veins in vivo.

The potent dose-dependent effects of the α_2 -receptor blocker yohimbine in the present study are in accordance with results

obtained in vitro (Steen et al 1984c), and confirms the potential significance of postjunctional α_2 -receptors in human veins.

Bobik and Anderson (1983) investigated the influence of sympathectomy on α_2 -receptor binding sites in canine blood vessels. Denervation of the lower abdominal aorta, renal and femoral arteries and femoral veins reduced vessel NA-content significantly, but had little effect on the concentration of α_2 -receptor binding sites or the affinity of ^3H -yohimbine for these sites. This might indicate that postsynaptic α_2 -receptors on blood vessels are located extrasynaptically and hence not primarily influenced by neuronally released NA (Langer 1982).

The differences in distribution of postjunctional α_1 - and α_2 -receptor subtypes between arterial and venous smooth muscle opens the possibility to influence the resistance and capacitance vessels selectively and this is of obvious clinical interest. Within the venous system regional variations in the distribution of α -receptor subtypes also seem to occur. However, to further verify this systematic studies have to be performed.

Thus, the results of the present study suggest that in vivo both α_1 - and α_2 -receptors are of functional significance. This is in contrast to previous in vitro findings (Steen et al 1984) and stresses the importance of comparison between information obtained in vitro and in vivo.

REFERENCES

- Andersson, K-E, B. Larsson & C. Sjögren. Characterization of the α -adrenoceptors in the female rabbit urethra. *Br J Pharmacol* 1984, 81:293-300.
- Bobik, A & W.P. Anderson. Influence of sympathectomy on α_2 -adrenoceptor binding sites in canine blood vessels. *Life Sci* 1983, 33 (4):331-336.
- Castenfors, J. & A. Felding. A simple technique for measuring changes in size of subcutaneous veins. In manuscript, 1984.
- Collier, J.G., C. Nachev & F. Robinson. Effect of catecholamines and other vasoactive substances on superficial hand veins in man. *Clin Sci* 1972, 43, 455-467.
- Dashwood, M.R. Central and peripheral prazosin binding: an in vitro autoradiographic study in the rat. *Eur J Pharmacol* 1982, 86 (1):51-58.
- De Mey, J.G. & P.M. Vanhoutte. Differences in pharmacological properties of postjunctional α -adrenergic receptors among arteries and veins. *Arch int Pharmacodyn* 1980, 244:328-329.
- De Mey, J. & P. M. Vanhoutte. Uneven distribution of postjunctional α_1 - and α_2 -like adrenoceptors in canine arterial and venous smooth muscle. *Circ Res* 1981, 48:875-884.
- Flavahan, N.A. & J.C. Mc Grath. Are human vascular α -adrenoceptors atypical? *J Cardiovasc Pharmacol* 1984, 6:208-210.
- Gillard, C., J. Boland & G. Calay. Short- and medium-term hemodynamic effects of prazosin in chronic refractory cardiac failure. *Arch Mal Coeur* 1982, 75 (4):469-477.
- Glusa, E. & F. Markwardt. Characterisation of postjunctional α -adrenoceptors in isolated human femoral veins and arteries. *Naunyn-Schmiedeberg's Arch Pharmacol* 1983, 323:101-105.

Holmberg, J.T., O. Thulesius, J.E. Gjores. Contractile response of isolated human vessels with special regard to stretch and storage. *Gen Pharmacol* 1983, 14:77-79.

Langer, S.Z. & N.B. Shepperson. Recent developments in vascular smooth muscle pharmacology: the post-synaptic α_2 -adrenoceptor. *Trends Pharmacol Sci* 1982 3:440-444.

Larsson, B., C. Sjögren, & K.-E. Andersson. Regional distribution of alpha-adrenoceptor subtypes in the female rabbit urethra. In manuscript 1984.

Ruffolo, R.R. Jr. Alpha-adrenoceptors. In: *Neuroreceptors in health & disease*, edited by J. Marwaha and W. Anderson. S. Karger AG, Basel, 1983.

Shoji, T., H. Tsuru & T. Shigei. A regional difference in the distribution of postsynaptic alpha-adrenoceptor subtypes in canine veins. *Naunyn Schmiedeberg's Arch Pharmacol* 1983, 324 (4):246-255.

Steen, S., T.V.C. Skärby, L. Norgren & K-E Andersson. Pharmacological characterization of postjunctional α -adrenoceptors in isolated human omental arteries and veins. *Acta Physiol Scand*, 1984 a, 120:109-116.

Steen, S., T. Sjöberg, T.V.C. Skärby, L. Norgren & K-E Andersson. Postjunctional α_1 - and α_2 -adrenoceptors mediating contraction in isolated human groin arteries and veins. Accepted in *Acta Physiol Scand*. 1984 b.

Steen, S., T. Sjöberg, T. Skärby, L. Norgren & K-E Andersson. The postjunctional α -adrenoceptors of the human saphenous vein. Accepted in *Acta Pharmacol Toxicol*, 1984 c.

Stevens, M.J. & R.F.W. Moulds. Heterogeneity of postjunctional α -adrenoceptors in human vascular smooth muscle. *Arch Int Pharmacodyn* 1981, 254: 43-57.

Timmermans, P.B.M.W.M. & P.A. van Zwieten. The postsynaptic α_2 -adrenoceptor. J. Auton. Pharmac. 1981, 1, 171-183.

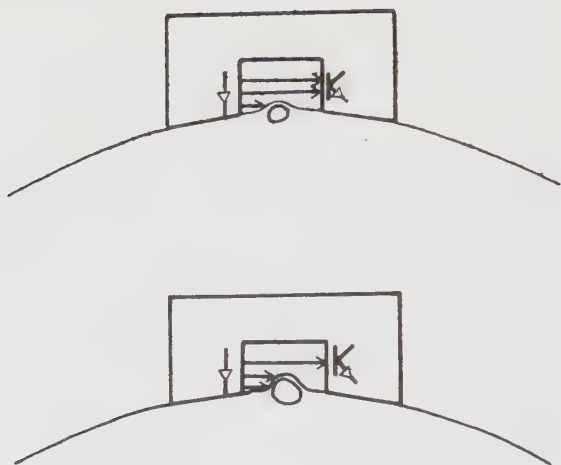


Fig 1. The device used to register changes in venous diameter. $\downarrow$ symbolizes a photodiode giving 940 nm infrared light through a 0.2 mm vertical slit. K symbolizes a photo-transistor measuring variations in light intensity.

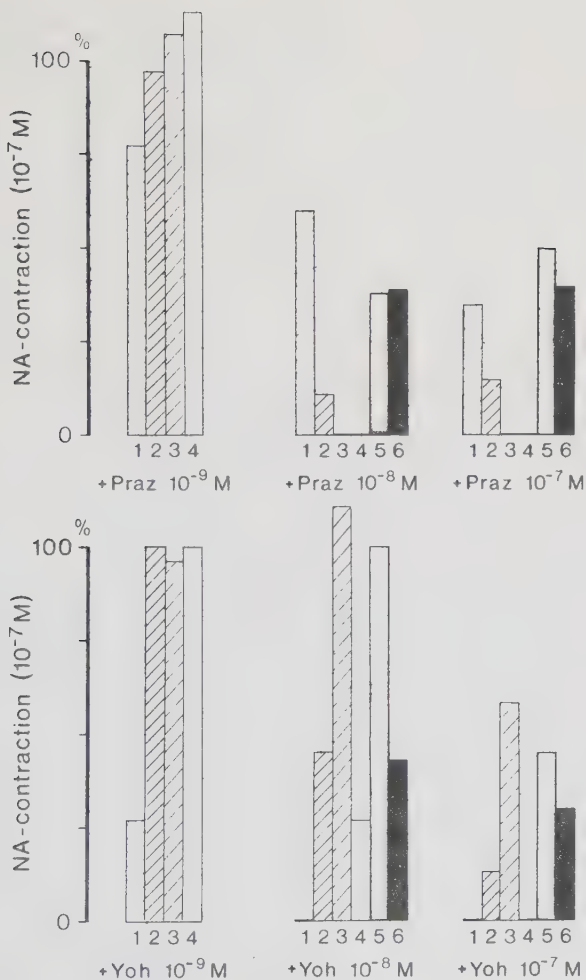


Fig 2. The contraction induced by 10^{-7} M noradrenaline (NA) in veins from six subjects (1-6) in presence of prazosin (Praz, upper panel) and yohimbine (Yoh, lower panel). All drugs were given with a continuous infusion rate of 0.3 ml/min. The contraction induced by NA in absence of antagonists was defined as 100 %.

HUMAN VASCULAR α -ADRENOCEPTORS IN THE ISCHEMIC LEG.

A study in vitro and in vivo.

Stig Steen , Per Alm , Karl-Erik Andersson , Trygve Sjöberg ,
Tor Skärby and Lars Norgren .

Departments of Surgery, Clinical Pathology and
Clinical Pharmacology, Lund University, Lund, Sweden.

Please send correspondence to:

Stig Steen, M.D.

Department of Surgery

Lund University

S-221 85 LUND

Sweden

Abstract

The α -receptor mediated contraction in crural arteries and veins obtained from patients with severe leg ischemia were studied *in vitro* and *in vivo*.

In vitro, the contractile capacity of the arteries (diam 0.4 - 1 mm) was generally poor and several arterial segments were unable to contract in response to the agonists tested (noradrenaline ($\alpha_1 + \alpha_2$), phenylephrine (α_1), clonidine (α_2)). Great inter- and intraindividual variations were seen, and repeated noradrenaline concentration - response curves showed a marked tendency to tachyphylaxis, making interpretation of antagonist experiments impossible. On the other hand, in the veins well reproducible contractions were obtained; the order of potency for the agonists tested was: clonidine = noradrenaline > phenylephrine. The α_2 -receptor blocker rauwolscine but not the α_1 -receptor blocker prazosin caused a concentration dependent rightward shift of the noradrenaline concentration - response curve. Morphological investigation of the arteries revealed slight to moderate atherosclerosis. In the veins no overt histopathological findings were found.

In vivo, noradrenaline caused a more than 70% reduction of femoral artery blood flow in all patients without systemic effects. The α_2 -receptor blocker yohimbine increased femoral blood flow in five patients, and decreased it in three, whereas prazosin increased it in all patients (by up to 150%). Papaverine increased the femoral blood flow by more than 350%. Yohimbine, prazosin and papaverine had all similar effects on the skin blood flow (measured by laser doppler technique), decreasing it in 6 patients and leaving it unchanged in 2 patients. Epidural anesthesia was unable to abolish the effects of noradrenaline. In spite of epidural anesthesia prazosin and papaverine increased femoral blood flow by up to 100% and 200% respectively.

INTRODUCTION

Several investigators have suggested that both α_1 - and α_2 -receptors can mediate contraction in vascular smooth muscle (for reviews see Timmermans & van Zwieten 1981, Mc Grath 1982, van Meel 1982, Ruffolo 1983).

Experiments on perfused hindlimb of rat (van Meel et al. 1983), rabbit (Madjar et al. 1980), cat (Gardiner & Peters 1982) and dog (Langer et al. 1980, Gardiner & Peters 1982, Llenas & Massingham 1983) indicate that the resistance vessels in these tissues are endowed with postjunctional α -receptors of both α_1 - and α_2 -subtype. Langer et al. (1980) when studying the auto-perfused hindlimb of the dog, suggested that the postjunctional α_2 -receptors were located predominantly outside the neuromuscular junction, whereas the postjunctional α_1 -receptors were to be found predominantly within the synaptic cleft. The observation leading to such a suggestion was that the response to electrical stimulation could be blocked by prazosin, but the drug was less effective in blocking the response to exogenously administered

noradrenaline (NA). The contraction mediating "extra-junctional" α_2 -receptors may perhaps contribute to peripheral resistance particularly in response to circulating catecholamines.

In man, however, evidence of the importance of contraction mediating postjunctional α_2 -receptors is scarce (van Brummelen et al. 1982, Elliott & Reid 1983). We have previously performed *in vitro* studies of the postjunctional α -receptors in vessels from the lower extremity of healthy subjects (Steen et al. 1984 b, c). The aim of the present investigation was to compare *in vitro* and *in vivo* information about the α -receptor mediated contraction in small arteries and veins in patients with severe leg ischemia due to atherosclerosis.

MATERIAL AND METHODS

Patients

The study was performed on 16 patients undergoing femoro-popliteal by-pass procedure because of severe ischemia due to atherosclerosis as judged from the angiographic findings. Eight were examined "in vitro" and eight in vivo. The protocol was approved by the Ethics Committee of Lund University and all patients gave written informed consent. For details of patient data see Table I.

Recording of mechanical activity in isolated small arteries and veins

During the groin and crural dissections small branches from the femoral vessels or from the posterior tibial vessels within the soleus muscle (diameter 0.4 - 1 mm for arteries and 1 - 2.5 mm for veins) were extirpated and immediately placed in chilled Krebs solution. The vessels were dissected free from fat and connective tissue under an operation microscope and care was taken to avoid stretching or other types of injury of the vessels. From each artery and vein, approximately 1 mm long ring segments were mounted in temperature-controlled organ baths filled with Krebs solution (for composition, see below). Cocaine, 10^{-6}M (for blockade of neuronal uptake), and propranolol, 10^{-7}M (to block β -adrenoceptors) were always present in the organ baths which were continuously bubbled with a mixture of 5% CO_2 and 95% O_2 giving a pH of approximately 7.4. The temperature was maintained at 37°C . The vessel segments were suspended between two L-shaped metal holders (0.2 mm in diameter), one of which was attached to a Grass FT03 transducer connected to a Grass polygraph for continuous recording of isometric tension. The position of the other holder could be changed by means of a movable unit allowing fine adjustments of vessel tension by varying the distance between the metal prongs (for further details see Högestätt et al. 1983). A passive tension of 8 mN was applied to both arteries and veins since separate experiments had shown that an optimum contractile response was obtained when the vessels were stretched to this tension.

After approximately one hour, when a steady level of 8 mN had been obtained, the vessels were contracted by exposure to a K^+ -rich buffer solution (for composition, see below). After two reproducible contractions (accepted variation less than 10%) had been evoked by the K^+ -rich solution, an agonist was added cumulatively and concentration-response (cr) curves were constructed on basis of data obtained. The K^+ -induced contraction was used as an internal standard in each segment, and each response was expressed as a percentage of the K^+ -induced contraction.

All cr-curves were plotted graphically and E_{max} , i.e. the maximum contraction obtained with an agonist, and EC_{50} i.e. the agonist concentration at which 50% of maximum contraction occurs, were calculated. pEC_{50} was defined as the negative logarithm of the EC_{50} -value.

Histological examination

Vessel segments were fixed in 10% formol (some after the experiments in the organ baths), dehydrated, embedded in paraffin, sectioned, stained and histopathologically evaluated.

Pharmacological tools

α_1 - and α_2 -receptor agonist: dl-noradrenaline hydrochloride (Sigma). α_1 -receptor agonist: l-phenylephrine hydrochloride (Sigma). α_2 -receptor agonist: clonidine hydrochloride (Boehringer Ingelheim). α_1 -receptor antagonist: prazosin hydrochloride (Pfizer). α_2 -receptor antagonists: yohimbine hydrochloride

(Sigma) and rauwolscine hydrochloride (Roth). "Non specific" vasodilator: papaverine sulphate (ACO). β -receptor blocker: dl-propranolol hydrochloride (ICI). Neuronal uptake blocker: Cocaine hydrochloride (Merck).

Solutions

The Krebs solution had the following composition (in mM): NaCl 119, NaHCO_3 15, KCl 4.6, CaCl_2 1.5, NaH_2PO_4 1.2, MgCl_2 1.2 and glucose 11. The K^+ -rich buffer solution had the following composition (in mM): KCl 124, NaHCO_3 15, CaCl_2 1.5, NaH_2PO_4 1.2, MgCl_2 1.2 and glucose 11.

In vivo studies

The registrations were done as the first moment of the operation. The first four patients were operated upon in epidural anesthesia, the last four in general anesthesia (pentothal, fentanyl, pancuronium, O_2 , N_2O).

In the first two patients the radial artery was cannulated for continuous registration of the arterial blood pressure. In the rest of the patients frequent measurements were done with a sphygmomanometer. ECG was continuously registered in all patients.

Electromagnetic blood flow measurements

A Cliniflow electromagnetic blood flowmeter (Model 601 D, Carolina Medical Electronics) was used. The common femoral artery and its branches were carefully dissected free from fat and connective tissue. A well fitting flow probe was then placed on the deep femoral artery close to the bifurcation. A zero level was obtained by occluding the arteries with vascular clamps.

Measurement of skin blood flow

variations with Laser Doppler technique

Periflux Laser Doppler Flowmeter PF1c (Perimed KB, Sweden) was used. A plastic probe head holder was placed by help of a double adhesive ringtape on the big toe or foot sole. If gangrene of the foot was present, the probe head holder was placed on the skin just proximal to the lateral malleolus. The measuring probe could then be placed near the skin and kept there in a steady position. The output signal (in volt) from the flowmeter is linearly related to the flux of red cells, i.e. the number of cells times their velocity. Laser light penetrates the measuring volume constituting approximately a hemisphere with a radius of about 1 mm in the outermost layer of the skin. The diffuse scattering of light in tissue makes the recorded flow value virtually independent of flow direction. All blood flow values were expressed in volt and recorded on a pen recorder. The zero level was obtained by placing the probe against a diffusely scattering plastic disc, (for further details of the method, see Nilsson et al 1980 a, 1980 b and Tenland 1982).

Examination procedure

All drugs were injected via a Venflon cannula (1.0 mm) placed in the common femoral artery. Only one bolus dose of each drug was given and the response was recorded on a pen recorder.

Preliminary experiments showed that 10 ml NA in a concentration of 10^{-5} M (=20.56 μ g) was an optimal dose to use, giving a good local response without significant systemic effects on blood pressure or heart rate. The dose chosen for yohimbine and prazosin was 10 ml of a concentration of 10^{-4} M (= 390.8 μ g and 418.8 μ g respectively). Papaverine was given in a bolus of 40 mg (10 ml in concentration 10^{-2} M). The drugs were given with 10 min intervals in the following order: NA, yohimbine, prazosin. Papaverine was given 5 min after prazosin. In this way the original blood flow level was reached before the next drug was given, except for papaverine which was given with some prazosin effect still present. This was done in order not to prolong the operation. Papaverine was used to estimate the capacity for vasodilatation in ischemic legs.

RESULTS

Functional studies of arteries in vitro

In the arteries the contractile response to all agonists tested was generally poor, and several vessel segments were unable to contract at all (Table II). Great interindividual variation

was seen (e.g. patient I and VII in Table II). The intra-individual variation (i.e. variation between segments from the same vessel) was also conspicuous (patient IV and V, Table II). Furthermore, repeated NA concentration - response curves showed a great tendency to tachyphylaxis (Fig 1), making the interpretation of antagonist experiments impossible.

Functional studies of veins in vitro

In contrast to arteries, well reproducible contractions were obtained in the veins (Fig 1). The order of potency for the agonists tested were: clonidine = NA > phenylephrine (Table II). Fig 2 shows an antagonist experiment using a small fragile vein (diam 1 mm) taken within the soleus muscle of a 77 year old woman (patient VIII in Table I & II) with diabetes mellitus and severe leg ischemia. As seen from Table II, strong contractions could be elicited with K^+ and still more with NA, which was especially potent (pEC_{50} 7.38); a small artery (diam 1 mm) taken from the same region in the same patient was unable to contract upon exposure to K^+ or NA. Rauwolscine, but not prazosin, caused a concentration dependent rightward shift of the NA concentration - response curve (Fig 2).

Histopathological findings

The vessel segments, representing small muscular arteries from ischemic legs, displayed small to moderate changes. Generally, the intima was focally thickened due to increased amounts of

subendothelial fibrous tissue in which a few cells with a foamy cytoplasm were found. Further, the internal elastic membrane had disappeared focally and in other areas there were only scattered remnants. The media showed increased fibrosis with a reduced number of smooth muscle cells. The cells were often hypertrophic and had nuclei which were slightly pleomorphic in shape and had a hyperchromic staining intensity giving an impression of slight degeneration. The morphologic pictures were compatible with slight to moderate atherosclerosis. In the vessel segments taken from small veins of the same patients no overt histopathological findings were noticed. Fig 3 b gives a typical example, showing a small muscular artery taken from patient I (Table I & II). This artery was unable to contract upon exposure to K^+ (124mM) or NA. For comparison is shown a small muscular artery taken from a 53 years old healthy woman (Fig 3 a).

In vivo results

In the peroperative experiments NA caused a more than 70% reduction of deep femoral artery blood flow (FBF; superficial femoral artery occluded by atherosclerosis) in all patients. There was a slight increase in arterial blood pressure, but no consistent changes in heart rate (Fig 4). The skin blood flow (SBF) measured as signal voltage, increased abruptly the first seconds after the bolus dose of NA had been given. When the maximum reduction in FBF was reached (after 30-90 s), SBF decreased rapidly (within 30 s) back to the basal level and below

in four of the patients (Fig 4). The NA effects disappeared gradually in 5 - 7 min.

Yohimbine increased FBF in five patients, and decreased it in three; no consistent changes in heart rate were observed, whereas there was a slight increase in blood pressure (Fig 4).

Prazosin and papaverine increased FBF in all patients. Prazosin caused a slight decrease in blood pressure and a slight increase in heart rate. Papaverine was injected too shortly after prazosin to make possible a proper evaluation of its effects. Both yohimbine, prazosin and papaverine had similar effects on SBF, decreasing it or leaving it unchanged (Fig 4).

DISCUSSION

The α -receptors on noradrenergic nerve endings regulating the release of transmitter (NA) were originally denominated α_2 -receptors in order to distinguish them from the classical post-junctional α -receptors which were called α_1 (Langer 1974). Later experience have confirmed that vascular prejunctional α -receptors generally are of the α_2 -subtype (see Skärby 1984). However, the types of postjunctional vascular α -receptors in different regions or in different types of vessel (i.e. arteries contra veins, large "conducting" arteries contra small "resistance" arteries or arterioles) have not yet been established. Information is largely lacking about the receptor function in vessels from patients

suffering from various pathological conditions. The main finding in the present in vitro study was that, in patients with severe leg ischemia due to atherosclerosis, leg veins behaved like groin- and saphenous veins in healthy subjects (Steen et al, 1984 b, c) whereas the arteries had a great tendency to tachyphylaxis and showed marked inter- and intraindividual variation in their contractile responses to K^+ and α -receptor subtype selective agonists (table II). However, the substantial effects of NA, prazosin and papaverine in vivo in all patients show that far from all vessels in ischemic legs are rigid non-reactive structures beyond pharmacological manipulation. The effects on FBF of prazosin and especially papaverine (in patient 1 - 4, fig 4) show that there is far more vasodilatation to be obtained than that caused by epidural anesthesia.

The selective α_2 -receptor antagonist yohimbine decreased FBF in patient 1, 4 and 8 and increased it in all other patients; a slight increase in blood pressure was seen in all patients. It may be speculated that in patients 1, 4 and 8 the α_2 -receptors situated prejunctionally on the sympathetic neurons predominated over those located postjunctionally and when yohimbine was given, the consequence was increased release of transmitter (NA) and subsequent vasoconstriction. The study performed by Elliott and Reid (1983) supports such an explanation. They demonstrated on healthy subjects an early transient increase in blood pressure and plasma NA after intravenous injection of a selective α_2 -receptor antagonist (RX 78 10 94).

Yohimbine increased FBF in patient 2, 3, 5, 6 and 7. This may be due to blockade of contraction mediating postjunctional α_2 -receptors. However, it cannot be excluded that the yohimbine concentration used to obtain this vasodilatation might have been too high (although diluted in vivo) to obtain a selective effect on α_2 -receptors. Some of the vasodilatation might therefore be attributed to blockade of α_1 -receptors.

In one of five patients tested the selective α_2 -receptor agonist clonidine elicited contraction of arteries in vitro (Table II), and this indicates that postjunctional α_2 -receptors may be present in atherosclerotic arteries from ischemic legs. Van Brummelen et al (1982) investigated the forearm blood flow with strain gauge pletysmography in five healthy volunteers and found that intraarterially injected yohimbine increased the forearm blood flow significantly (+194%) in a dose dependent way and that the α_2 -receptor agonist B-HT 933 decreased the blood flow in a dose dependent way. This response to yohimbine suggests a contribution of α_2 -receptor mediated vasoconstriction to basal vascular tone in healthy subjects.

In previous studies in vitro and in vivo (Steen et al, 1984 b, c, d) we have found that in groin and saphenous veins the contraction - mediating α -receptors of main importance are of α_2 -type although the presence of a population of α_1 -receptors can not be excluded. The in vitro data from the present study indicate that such a suggestion may be valid also for the veins in ischemic legs (Table II, Fig 2). The patients operated upon in epidural anesthesia had easily seen

subcutaneous veins. After the NA injection, these veins contracted heavily and "disappeared". In patient 1 who had especially dilated subcutaneous veins, the venoconstriction after NA was so pronounced that a furrow in the skin marking out the whole length of the long saphenous vein could be seen; when yohimbine was injected the same vein was seen dilating back to initial size in spite of a decrease in FBF.

A finding in the present study as well as in previous studies in man (Stevens & Moulds, 1981, Glusa & Markwardt, 1983, Arneklo-Nobin, 1983 and Steen et al, 1984 a, b) was that NA was more potent in veins than in arteries. There are differences in sympathetic innervation between veins and arteries (Owman 1980, Arneklo-Nobin 1983). The sympathetic innervation is generally best developed in arteries where the nerves are located in the adventitial layer, superimposed on the smooth muscle media layer without penetrating into the media layer itself; in veins the nerve terminals course in among the muscle cells. Veins generally seem to have more postjunctional α_2 -receptors than arteries (Steen et al. 1984 a, b) and these α_2 -receptors may be sensitive to circulating catecholamines because of their suggested extra-junctional location (Langer, 1980, 1981, 1982, Wilffert et al 1982).

Patients with manifest or threatening gangrene due to severe atherosclerosis or phlegmasia cerulea dolens are often stressed because of severe pain and anxiety, factors favouring high levels of circulating catecholamines. This may give an increased veno-

constriction critical for the run off. In these cases a selective vein dilating therapy (including α_2 -receptor blockade) might be of value.

REFERENCES

Arneklo-Nobin, B.: 1983. The white cold hand. Physiologic, angiographic and pharmacologic study on the hand circulation with special reference to Raynaud's phenomenon. Bulletin nr 37 from the Department of Surgery, University of Lund, Lund, Sweden.

Elliott, E.L. & J.L. Reid: 1983. Evidence for postjunctional vascular α_2 -adrenoceptors in peripheral vascular regulation in man. Clin Sci 65, 237 - 241.

Gardiner, J.C. & C.J. Peters: 1982. Postsynaptic α_1 - and α_2 -adrenoceptor involvement in the vascular responses to neuronally released and exogenous noradrenaline in the hindlimb of the dog and cat. Eur J Pharmacol 84, 189 - 198.

Glusa, E. & F. Markwardt: 1983. Characterisation of post-junctional α -adrenoceptors in isolated human femoral veins and arteries. Naunyn-Schmiedeberg's Arch Pharmacol. 323: 101 - 105.

Högestätt, E.D., K.-E. Andersson & L. Edvinsson: 1983. Mechanical properties of rat cerebral arteries as studied by a sensitive device for recording of mechanical activity in isolated small blood vessels. Acta Physiol Scand. 117: 49 - 61.

Langer, S.Z.: 1974. Presynaptic regulation of catecholamine release. *Biochem Pharmacol* 23: 1793 - 1800.

Langer, S.Z., R. Massingham, & N.B. Shepperson: 1980. Presence of postsynaptic α_2 -adrenoceptors of predominantly extrasynaptic location in the vascular smooth muscle of the dog hind limb. *Clin Sci* 59, 225s - 228s.

Langer, S.Z., N.B. Shepperson & R. Massingham: 1981. Preferential noradrenergic innervation of α_1 -adrenergic receptors in vascular smooth muscle. *Hypertension* 3, I-112 - I-118.

Langer, S.Z. & N.B. Shepperson: 1982. Postjunctional α_1 - and α_2 -adrenoceptors: Preferential innervation of α_1 -adrenoceptors and the role of neuronal uptake. *J Cardiovasc Pharmacol* 4, s 8 - s 13.

Llenas J. & R. Massingham: 1983. A comparison of the effects of verapamil and cinnarizine upon responses elicited by selective α_1 - and α_2 -adrenoceptor agonists in the autoperfused canine hindlimb. *Eur J Pharmacol*. 87, 53 - 59.

Madjar, H., J.R. Docherty & K. Starke: 1980. An examination of pre- and postsynaptic α -adrenoceptors in the autoperfused rabbit hindlimb. *J Cardiovasc Pharmacol* 2: 619 - 627.

McGrath, J.C: 1982. Evidence for more than one type of post-junctional α_2 -adrenoceptor. *Biochem Pharmacol* 31: 467 - 484.

Nilsson, G.E., T. Tenland & P.Å. Öberg: 1980 a. A new instrument for continuous measurement of tissue blood flow by light beating spectroscopy. *IEEE Trans Biomed Eng* BME-27, 12-19.

Nilsson, G.E., T. Tenland & P.Å Öberg: 1980 b. Evaluation of a laser doppler flowmeter for measurement of tissue blood flow. *IEEE Trans Biomed Eng* BME-27, 597 - 604.

Owman, C.: 1980. Vascular autonomic nerves and corresponding receptors, with particular reference to neurogenic mechanisms of vasodilation. *Progr. Pharmacol.* 3: 47 - 68.

Ruffolo, R.R. Jr.: 1983. Alpha-adrenoceptors. In: *Neuroreceptors in Health & Disease*, edited by J. Marwaha and W. Anderson. S. Karger AG, Basel.

Skärby, T. 1984. Pharmacological properties of prejunctional α -adrenoceptors in isolated feline middle cerebral arteries; comparison with the postjunctional α -adrenoceptors. Accepted for publication in *Acta Physiol Scand*.

Starke, K. & S.Z. Langer: 1979. A note in terminology for pre-synaptic receptors. In: *Presynaptic receptors* (ed. S.Z. Langer, K. Starke & M.L. Dubocovich), pp. 1 - 3. Pergamon Press, Oxford.

Steen, S., T.V.C. Skärby, L. Norgren & K.-E. Andersson: 1984 a. Pharmacological characterization of postjunctional α -adrenoceptors in isolated human omental arteries and veins. Acta Physiol. Scand. 1984 a, 120: 109 - 116.

Steen, S., T. Sjöberg, T.V.C. Skärby, L. Norgren & K.-E. Andersson: 1984 b. Postjunctional α_1 - and α_2 -adrenoceptors mediating contraction in isolated human groin arteries and veins. Accepted in Acta Physiol Scand.

Steen, S., T. Sjöberg, T. Skärby, L. Norgren & K.-E. Andersson: 1984 c. The postjunctional α -adrenoceptors of the human saphenous vein. Accepted in Acta Pharmacol Toxicol.

Steen, S., J. Castenfors, T. Sjöberg, T. Skärby, K.-E. Andersson & L. Norgren: 1984 d. Effects of α -adrenoceptor antagonists on the human saphenous vein in vivo. In manuscript.

Stevens, M.J. & R.F.W. Moulds: 1981. Heterogeneity of postjunctional α -adrenoceptors in human vascular smooth muscle. Arch. Int. Pharmacodyn 254: 43 - 57.

Tenland, T.: 1982. On laser doppler flowmetry. Methods and microvascular applications. Linköping University Medical Dissertation No 136.

Timmermans, P.B.M.W.M & P.A. van Zwieten: 1981. The postsynaptic α_2 -adrenoceptor. J. Auton. Pharmacol 1: 171 - 183.

Van Brummelen, P., P. Vermey, P.B.M.V.M. Timmermans & P.A. Van Zwieten: 1982. Preliminary evidence for a postsynaptic α_2 -adrenoceptor in the vasculature of the human forearm. Proceedings of the P.B.S. 8-10 Sept.

Van Meel, J.C.A.: 1982. The vascular postjunctional α_2 -adrenoceptor. Rodopi Amsterdam. Thesis.

Van Meel, J.C.A. P.B.M.W.M. Timmermans & P.A. Van Zwieten: 1983. α_1 - and α_2 -adrenoceptor stimulation in the isolated perfused hindquarters of the rat: An In Vitro Model. Cardiovasc Pharmacol 5: 580 - 585.

Wilffert, B., P.B.M.W.M. & P.A. Van Zwieten: 1982. Extrasynaptic location of alpha- and noninnervated beta- adrenoceptors in the vascular system of the pithed normotensive rat. J. Pharmacol Exp. Ther. 221, 762 - 768.

Patient	Age/sex	Anaesthesia	Run off	Gangrene/ rest pain	Basal heart rate (min ⁻¹)	Basal systolic pressure (mm Hg)	Basal femoral blood flow (ml/min)	Basal skin flow (volt)	Drug treatment
I	59 ♂	EA	2/3	-/+	72	140			
II	92 ♂	EA	2/3	-/+	92	190			
III	53 ♂	GA	2/3	+/+	76	140			Digoxin, Nitro- glycerin, Furosemid
IV	52 ♂	EA	2/3	+/+	80	140			
V	42 ♂	GA	3/3	-/-	74	140			
VI	58 ♂	EA	3/3	-/+	88	130			Metoprolol, Spironolacton Chlortalidon
VII	57 ♂	EA	2/3	-/+	70	160			
VIII	77 ♀	EA	2/3	-/+	96	170			Verapamil, Spironolacton Insulin
1	62 ♂	EA	3/3	-/+	70	95	100	0.02	
2	71 ♂	EA	2/3	-/+	82	140	210	0.4	
3	77 ♀	EA	2/3	-/+	72	115	80	0.2	Spironolacton, Furosemid
4	76 ♂	EA	1/3	+/+	64	115	140	0.25	Digoxin, Verapamil, Furosemid
5	76 ♂	GA	2/3	-/+	70	90	80	0.03	Digoxin, spiro- nolacton, Nifedipin Warfarin
6	72 ♀	GA	1/3	+/+	72	150	70	0.08	Prednisolon
7	77 ♀	GA	2/3	+/+	64	115	70	0.15	Digoxin, Furosemid
8	68 ♂	GA	2/3	-/+	84	160	28	0.04	

Table I

Data on the patients operated upon because of severe leg ischemia. Roman numerals denote patients involved in experiments in vitro. Arabic numerals denote patients involved in the peroperative investigations. EA = Epidural anaesthesia. GA = General anaesthesia. Run off denotes number of patent arteries (anterior or posterior tibial and peroneal) as seen on the angiograms. All patients had atherosclerotic occlusions of the superficial femoral artery. Three patients (II, V, VII) were known hypertonics, three patients (3, 4, 5) had a history of previous myocardial infarction, only one patient (VIII) had diabetes and one patient (5) was under treatment because of cerebrovascular disease.

Table II. Effects of α -adrenoceptor agonists on isolated crural arteries and veins obtained from patients with severe leg ischemia. The contractile responses are expressed as percentage δ of the K^+ (124 mM) -induced contraction.

	K^+ (mM)	Noradrenaline (α_1 & α_2) pEC_{50}	K^+ (mM)	Phenylephrine (α_1) f_{max} (% of K^+)	pEC_{50}	K^+ (mM)	(Clonidine) (α_2) f_{max} (% of K^+)
I	0	0					
II	0	0	0	0		0	0
III	5	169	29	99	4.90	52	0
IV	0	0	2	6	5.44	12	0
	12	0					
	6	32					
V	0	20	17	0		22	0
	12	0					
VI	9	60					
VII	31	34	17	33	3.51	22	14
VIII	0	0					6.54
I	35	114	40	100	5.45	75	6.30
II	37	53	26	70	5.16	26	14
III	17	119					5.62
VI	12	91	17	91	5.32	29	5.49
VIII	17	124					
	23	109					
	19	135					

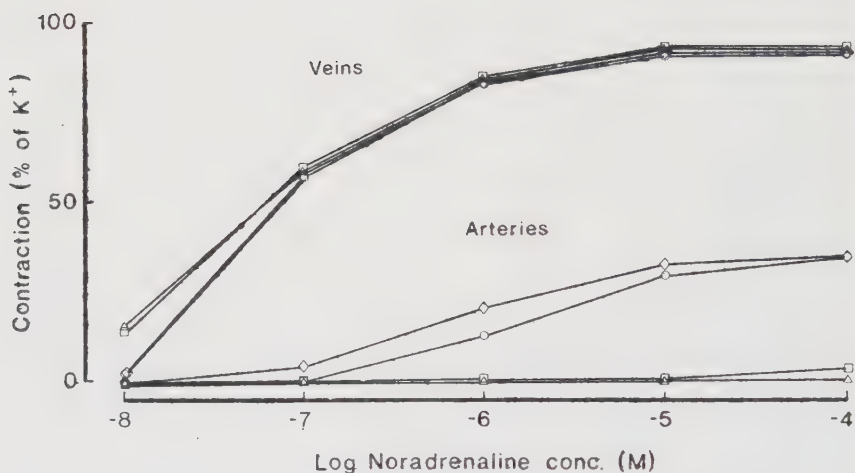


Fig 1. Repeated ($\diamond$, $\circ$, $\square$, Δ) noradrenaline concentration - response curves in isolated small artery and vein segments (about 1 mm diam) obtained from within the soleus muscle of a 59 year old man with severe leg ischemia (patient I, Table I & II). The contractile responses are expressed as percentage of the K^+ (124 mM)-induced contraction.

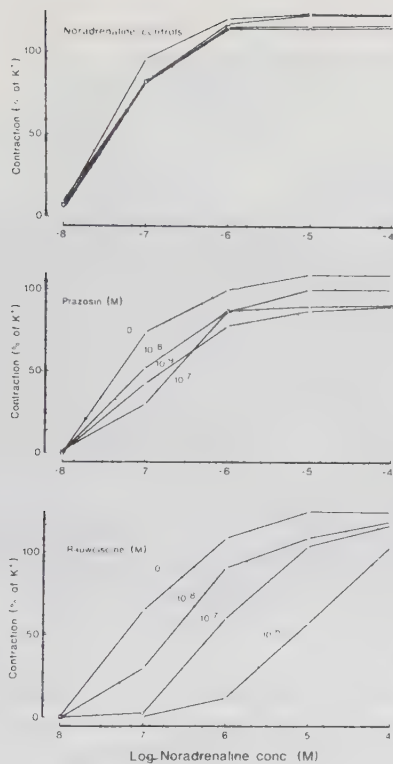
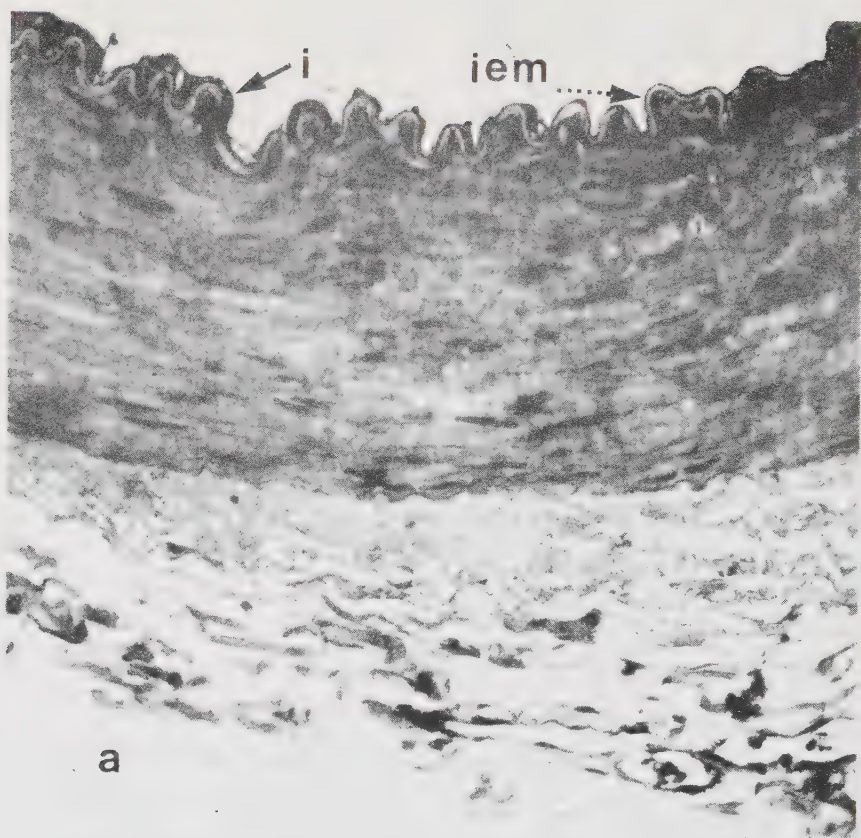


Fig 2: An antagonist experiment performed on a small vein segment (about 1 mm diam) obtained from within the soleus muscle of a 77 year old woman with severe leg ischemia and diabetes mellitus (patient VIII, Table I & II). Three segments from the same vein specimen were investigated in parallel, using the first segment as a control (upper panel) and the second and third segment for testing the effect of increasing concentrations of the α_1 -receptor blocker prazosin (middle panel) or the α_2 -receptor blocker rauwolscine (lower panel). The responses to noradrenaline (stimulating both α_1 - and α_2 -receptors) are expressed as percentage of the K⁺ (124 mM)-induced contraction.



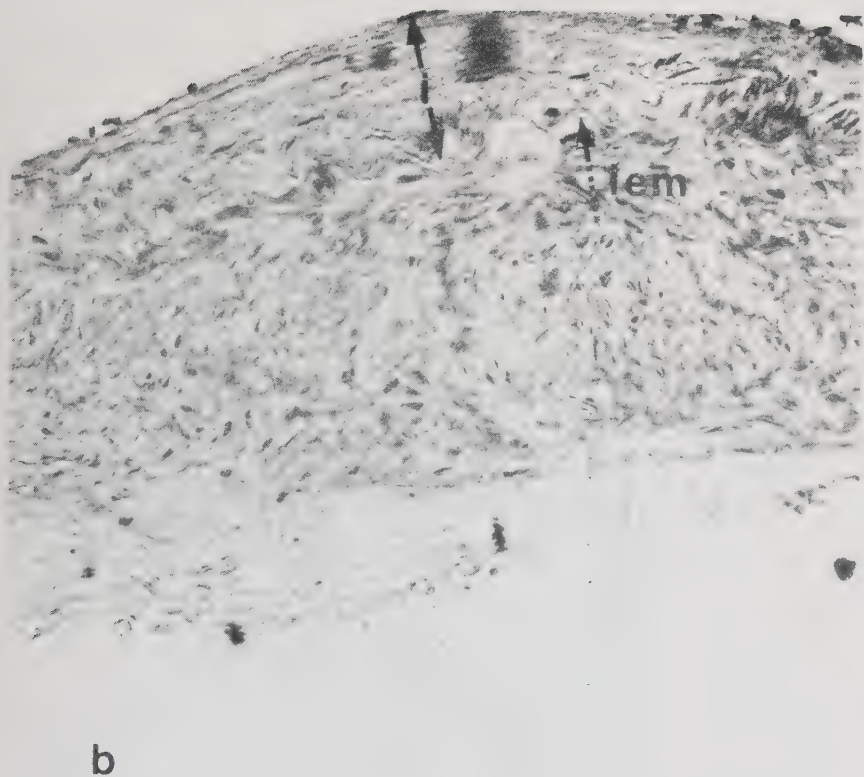


Fig 3. a.Branch of a small muscular artery from the groin region of a 53 years old healthy woman. The intimal layer ($i \rightarrow$) is thin. The internal elastic membrane ($iem \dashrightarrow$) is generally well preserved. b.Branch of a small muscular artery from the soleus muscle of a 59 years old man with severe leg ischemia (patient I, Table I & II). Compared to Fig 3 a the intimal layer ($\leftarrow i \rightarrow$) is thicker and the internal elastic membrane ($iem \dashrightarrow$) is only focally preserved. Light micrographs. Mac Manus x 225.

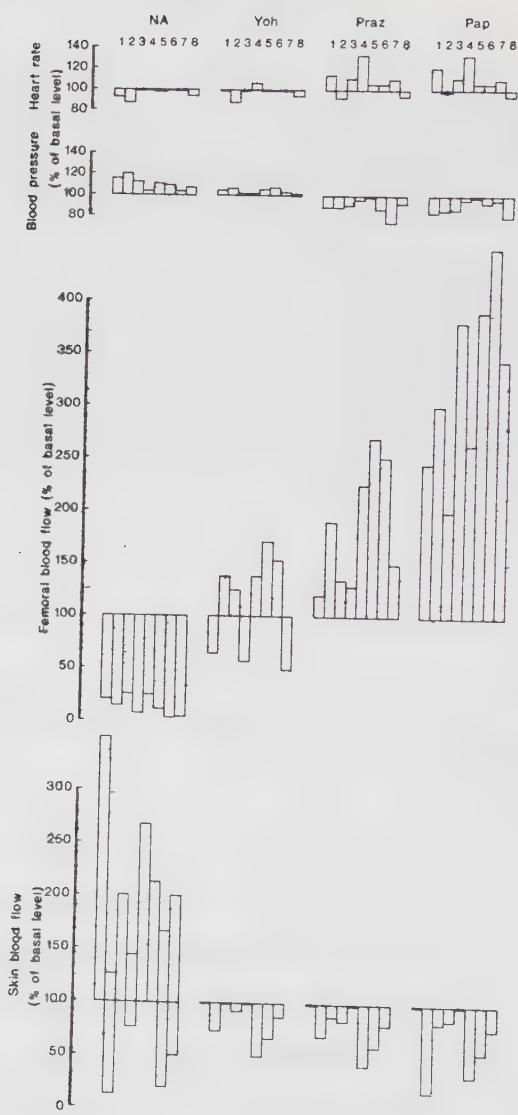


Fig 4. Registration of heart rate, systolic blood pressure and leg blood flow before and after intraarterially administered vasoactive drugs in 8 patients (1-8) undergoing femoro-popliteal by pass procedure because of severe ischemia. Epidural anesthesia was used in patients 1 - 4 and general anesthesia in patients 5 - 8. Femoral blood flow was measured with electromagnetic flow-meter by placing the flow probe on the deep femoral artery close to the bifurcation. Variation in skin blood flow (in signal voltage) was registered by laser doppler technique. The drugs were given in the common femoral artery during 10 s in the following order: noradrenaline (NA, $10 \text{ ml } 10^{-5} \text{ M}$), yohimbine (Yoh, $10 \text{ ml } 10^{-4} \text{ M}$), prazosin (Praz, $10 \text{ ml } 10^{-4} \text{ M}$) and papaverine (Pap, $10 \text{ ml } 10^{-2} \text{ M}$). Peak responses are given (in % of basal levels).

